NATURAL HISTORY of CORONARY ATHEROSCLEROSIS

Constantin Velican
Doina Velican

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Natural History of Coronary Atherosclerosis

Authors
Constantin Velican, M.D., Ph.D.
Doina Velican, M.D.
Institute of Internal Medicine
Colentina Hospital
Bucharest, Romania

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PREFACE

Based on the experience gained during more than 2 decades of uninterrupted work on the natural history of human coronary atherosclerosis, we have attempted in this book to integrate our results with those which exist in the available literature. The personal character of the material included in many chapters or sections of these chapters reflects this accumulated experience in the field of atherosclerosis research.

The first major objective of this book is to present the natural history of coronary atherosclerosis as it takes place in the living population. This natural history, as analyzed in this work, includes:

1. A large variety of atherogenic mechanisms able to give rise to early lesions during childhood, adolescence, and adulthood
2. Six types of early lesions with different light microscopic features and prevalences in successive age groups and segments of the coronary arterial tree
3. Particular patterns and rates of progression of these early lesions toward advanced ones, with the occurrence of some advanced lesions in only 1 to 3 years
4. The development of lesions of possible clinical significance in both major coronary arteries and main branch vessels
5. The existence of fibronecrotic and fibrohyaline plaques on which repeated cycles of thrombus formation and disintegration take place, associated with peripheral embolization
6. A close link between this dynamic pathology of advanced coronary atherosclerotic lesions on the one hand, silent myocardial ischemia, and the clinical spectrum of coronary heart disease, on the other hand

The second major objective of this book is to show that the natural history of coronary atherosclerosis, once the domain of only a few pathologists and experimentalists, has now attracted the interest of scientists from a wide variety of specialities. Of note is the penetration of atherosclerosis research into the spheres of cell biology and genetics; there is also ample promise that the field of biomechanics, through collaborative medical-engineering effort, will establish itself as an essential and integral part of atherosclerosis research. These new orientations are associated with the invalidation of many old concepts with the evolution of a new language and the development of new insights.

The third major objective of this book is to emphasize that no single etiologic agent and pathogenetic mechanism have been clearly implicated as yet in the onset of early lesions in human coronary arteries. Also, no useful result was reached during an entire century by continuing to put forward one theory to the exclusion of others. In order for one theory to be accepted, it is not necessary that the others must be rejected. Rather, all results and hypotheses available should be taken into consideration, since they try to explain the multifactorial origin of human coronary atherosclerosis and the large diversity of pathogenetic mechanisms, as well as of genetic and environmental influences. Our aim is to look at the current evidence together with the enthusiasts who frequently used the term "causal significance" and with the skeptics who do not use this term, as well as with those who obtain their information from experimental research, from intra- and interpopulation studies, or from dietary manipulations. We hope that the material presented in this work will offer to all these investigators a source of information for reinforcing, or, on the contrary, for revising their concepts.

The fourth major objective of the book is to persuade the reader that there are many traps to fall into in a study on the natural history of coronary atherosclerosis. Some examples are presented which demonstrate that sometimes scientists could not avoid the dangerous trap of oversimplification in an attempt to gain more clarity; this usually masks the real complexity of the phenomena, some aspects or mechanisms being overemphasized, other minimized,

or even overlooked. Additional traps may be some conclusions on human coronary atherosclerosis based on inadequate animal models. Another trap is the belief that lipids are the *sine qua non* of human coronary atherosclerosis and that each pathologist must attempt a direct assault on macroscopic sudanophilia. Finally, an important source of error could reside in the fact that many investigators are not well versed in the variations of the coronary arterial tree anatomy. In too many papers, the important variability of the coronary branching pattern as related to atherosclerotic involvement is neglected, as well as the presence of lesions of possible clinical significance in the main branch vessels and in the vessels supplying the conduction system.

The privilege to write a book on the natural history of human coronary atherosclerosis implies many responsibilities, starting with due recognition to major contributions past and present. The number of studies in this field is overwhelming and it would have been impossible in a book of this size to do justice to all who have contributed to this enormous literature. Many good studies are conspicuously absent simply because of space limitations, but we believe that the references are sufficient in number and range to provide a useful guide for those who seek further information.

We hope that this volume, because of in-depth reviews, critiques of theories and methods, and abundant personal experience, will be of interest to cardiologists, pathologists, angiographers, pediatricians, epidemiologists, physiologists, biochemists, biophysicists, and medical students, as well as to all physicians who attempt to prevent and treat coronary atherosclerosis and coronary heart disease.

THE AUTHOR

Constantin Velican, M.D., Ph.D., is Head, Department of Pathology and Histochemistry, Institute of Internal Medicine, Colentina Hospital, Bucharest, Romania. He is a graduate of the Faculty of Medicine, University of Bucharest (1943).

Dr. Velican was a recipient of the Academy Prize for Medicine in 1944, 1964, and 1975. He is a member of many societies of medicine and biology and of several editorial boards. He has done research in connective tissue histochemistry and pathology and in human coronary and cerebral atherosclerosis and promoted preclinical orientation in pathology. He has published more than 300 research papers (23 in *Atherosclerosis* from 1967 to 1986) and 12 books, including *Band VIII/2, Handbuch der Histochemie (Macromolecular Changes in Artherosclerosis*, Gustav Fischer, Stuttgart, 1974).

His current major research interests include the natural history of atherosclerosis, as related to anatomical branching pattern, and the histoarchitecture of human coronary arteries.

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Chapter 1

OVER A CENTURY OF RESEARCH ON THE NATURAL HISTORY OF CORONARY ATHEROSCLEROSIS

I. THE PRESSING NEED FOR ADEQUATE TERMS AND DEFINITIONS

A. Historical Survey

Coronary atherosclerosis seems to be widely distributed in time, since it has been alternatively described as a "disease of antiquity" and a "twentieth century disease".[1]

According to some deductions, paleolitic human beings would have ingested about 600 mg cholestrol each day.[2] From 1955 to 1956, the incidence of myocardial clinical manifestations due to coronary atherosclerosis in 55- to 59-year-old men in the U.S. was 750/100,000 and their average cholesterol intake was also about 600 mg/day.[3]

Coronary atherosclerosis was common in ancient Egypt, as demonstrated by the study of many Egyptian mummies ranging over 2000 years from 1500 B.C. to 520 A.D.[4] Analytical investigations revealed calcified, ulcerated, and thrombotic plaques similar to those described in the human pathology of the 20th century.[5,6] The marquise of Tai (China), approximately 2100 years ago according to carbon dating, showed generalized atherosclerosis and severe involvement of the coronary arteries with stenotic plaques in the left anterior descending artery which encroached upon more than 75% of the vessel lumen.[7] A frozen mummy from Alaska dated approximately 400 A.D. also exhibited extensive coronary atherosclerosis.

The first attempt to define in morphological terms arterial wall changes considered to represent atherosclerotic lesions are attributed to old Greek writers who used the term "athera". Aristotle and his associates reported the first known data on age of pathologically related changes in large vessels and the physician Galen of Pergamos in Asia Minor (120 A.D.) seems to be the first scientist to employ the term "coronary arteries". Leonardo da Vinci (1452 to 1519) illustrated the close relations between these vessels and the heart, indicating in many anatomical drawings the origin of the coronary arteries and some changes which occur with aging. Particularly in his work dealing with the anatomy of the elderly, he emphasized the important narrowing of the coronary arteries that may lead to restriction of the passage of the blood which nourishes the heart.

The relationships between coronary artery narrowings and anginal pain were suggested for the first time by Vesalius (1555), a professor of anatomy in Padua, Italy. The particular value of these observations increased when Harvey (1645) accurately showed the pumping action of the heart and the presence of many intramyocardial vessels. These were more clearly demonstrated by Lower (1671), using fluid injection techniques, and by Ruysch (1704), who employed a corrosive method able to reveal small intraventricular branches of the major coronary arteries. During the same period, Vieussens (1706) demonstrated, with the aid of saffron injected into the coronary arteries, the existence of connections between these vessels and the cardiac chambers. On the other hand, Thebesius (1708), using air injected through the coronary sinus, revealed the existence of connections between the coronary veins and cardiac chambers. Only by the end of the 19th century was important progress made in this field of investigation, when Baumgartner (1899) used the coronary radiography on postmortem material and Spaltenholtz developed methods of clearing the heart tissue in organic solvents, so that injected vessels could be seen beneath the surface. Of particular importance for the natural history of coronary atherosclerosis was the investigation carried out by Merkel (1909). This was the first attempt to use radioopaque substances to detect pathological changes in the coronary arteries.

Beginning with the first half of the 18th century, interest in the significance of atheromas

was renewed by many pathologists, particularly by the works of Brunner (1729) and Haller (1755). This interest increased when Heberden (1772) presented the first clinical data on a heart disease called angina pectoris. Particular emphasis was placed on exertion, which is considered to appear as a result of pathologic changes in the coronary arteries. The first written autopsy protocol of a patient with angina pectoris is attributed to Hunter (1775), who found that the coronary arteries were transformed into a piece of bone. A clear connection between coronary artery alteration and the clinical symptoms which manifested as anginal pain also appeared in the works of Burns (1809). Moreover, Bichat (1801) offered new detailed descriptions of these alterations and Hodgson and his associates (1815) were able to perform the first chemical analysis of an atherosclerotic coronary artery. Also of note is Hunter's observation that emotional upheavals and stress may precipitate coronary death. During the same period the first indications concerning the prevention of angina pectoris were offered by Fothergill (1712 to 1780) who forecasted the role of diet and psychosocial factors in the onset of this disease. In the same sphere of angina pectoris research it may be of interest to remember that in 1867 the British pharmacologist Brunton showed that amyl nitrate administered during an attack of angina pectoris could shorten the paroxysm or at least reduce its severity. Even more important was the discovery in 1779 by Murrell of the particular clinical value of nitroglycerin to relieve anginal pain.

A new line of research was opened by the observations of Hunt (1899) that right and left sympathetic nerves have different effects on the heart rate, contractility, and repolarization. These observations began innumerable attempts to prevent and treat angina pectoris. Of particular importance was the sympathectomy proposed by Frank (1899), the first cervical sympathectomy being performed by Jonesco (1920) in Bucharest, with dramatic relief of the patient's symptoms.[8] This operation was followed by an era of various denervation procedures and by new attempts to investigate the pathogenetic mechanisms, particularly the existence of an imbalance between the workings of the heart and its blood supply.[9]

The Italian anatomist and surgeon Scarpa (1752 to 1823) showed that atheroma may give rise to aneurysms and suggested that fatty infiltration and intimal thickening are mainly involved in this evolution. Other investigators tried to differentiate atheroma from other pathologic changes of the artery wall Lobstein proposed the term "arteriosclerosis" in 1833 to designate intimal thickening, medial sclerosis, medial hyalinization, and less of the elasticity of the arterial wall.[10] Likewise, Mönckeberg, in 1903, described medial calcification as a separate pathologic entity.

The complexity of the arterial pathologic changes was also emphasized by Virchow.[11-14] He stressed the nodular character of certain intimal proliferations (endarteritis chronica sive nodesa), the particular role of inflammation, and observed that a "mucous substance" accumulated within the arterial intima in the early stages of proliferative lesions. A similar mucuous substance was detected by Westphal (1880) in mast cells. Several investigators were able to correlate the intense metachromasia of the intimal ground substance with the susceptibility of some arterial regions to atheroma development.[15-18]

Virchow (1856 and 1864) was also impressed by the abundance of lipid accumulations within atheroma-like lesions, particularly fatty bodies showing a double contour. These fatty bodies were also found in degenerating tissues in the form of small spheres and were called "myelin bodies". In 1857, Mettenheimer assumed that myelin-like bodies are a characteristic feature of human atheromas and suggested that these small spheres include cholesterol from the flowing blood.[19] Of note is the observations of (1) Adami and Aschoff (1906), who revealed an important amount of sudanophil and anisotropic material in atheroma-like lesions and (2) Dietrich (1910), who was able to identify both cholesterol and phospholipids in the material.[20,21] More analytical analyses were carried out by Windaus (1910). The results showed that there were 6 to 7 times more free cholesterol and 20 to 26 times more bound cholesterol in atheromas than in neighboring normal intima.[22] Thus, the view of many famous

pathologists, such as Virchow (1856), Ribbert (1904), and Aschoff (1908), that during atheroma formation there is a progressive cholesterol accumulation within the lesion was supported by the first chemical studies.[13,23,24] Whereas in the first half of the 19th century studies on arterial pathology were focused almost exclusively on sclerosis, calcification, necrosis, and thrombosis without any particular interest for biochemists, in the second half the scientists' attention was progressively centered on intraarterial lipid accumulation. This new orientation fostered the family of theories (usually called "lipid hypotheses") involving the permeation of lipids from the lumen into the arterial intima and the failure of arterial wall homeostasis to keep the intima free of lipid deposits. Virchow was able to differentiate reversible intimal fatty accumulations which occur in children and adolescents and atherosis present in mature adults. A pathogenetic relationship between these two types of lesions was suggested by Jores in 1903, the subendothelial fatty streak being presented as an early stage of atheroma formation. This view was further developed by Askenazy (1907) and by Klotz and Manning (1911).

A very important theory of atheroma formation was advanced by Rokitansky in 1852, who postulated that this lesion occurs as a result of the organization of intravascular fibrin deposits.[25] This "thrombogenic theory" has been and continues to be handicapped by a lack of adequate methods to detect fibrin within human arterial intima and also by that of convenient animal models. Nevertheless, the interest in the role of thrombosis in arterial pathology increased considerably, when in 1859 Malmsten revealed a positive correlation between coronary thrombosis and coronary atheromas, myocardial degeneration, and myocardial rupture. This view was supported by the subsequent studies of Weigert (1880), Ziegler (1882), and Benke (1890), which demonstrated a close association between coronary atheromas and coronary occlusions produced by thrombosis.[26-28] Note also the account published in 1878 by Hammer of "a case of thrombotic occlusion of one coronary artery of the heart", since it includes the term "acute coronary thrombosis" which became largely accepted by clinicians and pathologists.[29]

A starting point along a different line of research on the natural history of coronary atherosclerosis was an additional hypothesis formulated by Rokitansky (1852) and largely developed by Rindfleisch (1870). According to this hypothesis, atheromas occur at sites in arteries which experience the full stress and impact of blood. Many investigators showed a particular interest in this suggested role of hemodynamics in the genesis of human atheromas. This attitude was reflected in studies which revealed that lesions are localized within regions of sharp curvatures and at branching points, and that the first 2 to 4 cm of the major coronary arteries and particularly of the left anterior descending artery are the vascular areas most susceptible to atheroma formation and to the onset of thrombotic occlusions. Although it was not possible to explain the pattern of coronary involvement by fatty streaks and atheroma-like lesions only from the viewpoint of hemodynamics, significant features of this pattern were progressively revealed. Consequently, the attention of certain investigators was centered on the interactions of fluid mechanics with the biology of the arterial wall.

By the end of the 19th century, Marie (1896) presented the first study on myocardial infarction and its clinical consequences, including aneurysm and rupture of the heart. Data on myocardial infarction as an end stage in the natural history of coronary atherosclerosis also apeared in the study of Obrastzov and Straschesko (1910). They emphasized the role of physical exertion and emotional stress in sudden cardiac death and presented selected cases in which a diagnosis of coronary thrombosis was made while the patients were still alive. Herrick (1912) was also able to report a diagnosis of myocardial infarction in the living patient. The interest of both clinicians and pathologists in the relationships between coronary pathology and myocardial clinical manifestations increased considerably when Pradee (1920) localized in living patients myocardial infarcts produced by coronary thrombosis. His article "An Electrocardiographic Sign of Coronary Obstruction" opened a new

era in the study of the natural history of coronary atherosclerosis. Strongly related to the thrombogenic theory of atheroma formation and myocardial infarct production are the observations that injuries to the arterial wall are followed by platelet adhesion and disintegration.[30-32] Bizzozero (1882) was the first investigator who demonstrated, in a light microscopic study, the presence of platelet aggregation induced by an experimental mechanical injury of an animal artery.[30] Emphasis was gradually placed on endothelial injury, and it may be of interest to recall that the endothelium was recognized in 1841 by Henle, but the term is attributed to His (1865). As early as 1866, Langhans presented the first "en face" preparations from the endothelium and intima, an adequate method to detect the role of injuries in atheroma formation. The importance of these injuries considerably increased when Weigert (1880) demonstrated that coronary thrombosis could be involved in both diffuse myocardial fibrosis and myocardial infarction.[26]

The first attempt to produce in laboratory animals arterial lesions similar to those detected in human arteries is attributed to Josué (1903). After adrenalin injection, he observed necrosis of the arterial media, followed by reparative changes, calcification, and fibrosis.[33] Josué's experimental model is of particular importance since it represents the starting point of all studies which try to demonstrate the atherogenic potential of various types of modern life stresses, acting by catecholamine release.

A different type of experimental model was proposed by Ignatowsky (1908), who showed that a particular type of diet may induce arterial changes in laboratory animals. By feeding rabbits a diet containing milk, meat, eggs, and other animal products, he was able to produce many pathologic changes in the aorta.[34] Stuckey (1912) also revealed that a diet high in egg yolk caused arterial lesions, whereas one rich in sunflower seed and fish oil was not followed by similar pathologic changes.[35] The most important experimental model of atherosclerosis was proposed by Anitschkow and Chalatow (1913), who demonstrated that the addition of "pure" cholesterol to a rabbit's diet led to aortic changes similar to human fatty streaks. This because the basic experimental model of atherosclerosis and suggested that cholesterol might represent the etiologic agent of human disease. In the light of historical perspective, Anitschkow's model is the first attempt to demonstrate the importance of blood lipid changes before and during the onset of fatty streak-like lesions; it has become the first standard test for the study of dietary constituents, hypolipidemic drugs, and of other agents that can influence the course of experimental lesions. It permitted controlled studies of the reversibility of lipid-rich lesions. The onset of fatty streak-like lesions after cholesterol-rich diets was considered by many experimentalists to demonstrate clearly that hypercholesterolemia is both necessary and sufficient to produce lesions. Accordingly, the process of lipid accumulation within the arterial wall is presented as a primary event and a consequence of prolonged hypercholesterolemia.[36,37]

Anitschkow has also stressed the view that human type advanced atheromas develop in the rabbit arteries, not only as a result of exposure to hypercholesterolemia for a sufficiently long period of time, but also if the arteries are slightly injured.[38,39] The role of injuries in the occurrence of "susceptible sites" for lipid accumulation was clearly demonstrated in one of Anitschkow's first reports on experimental hypercholesterolemia. He proposed a theory of atherogenesis based on the concept of "pluricausality". According to this concept, arterial wall injury is the first causative factor in the onset of lesions, whereas the resulting necrosis, inflammation, and other repair features may favor lipid accumulation at the site of injury. Injuries to the arterial wall produce not only accelerated onset of lipid-rich lesions, but also the development of such lesions in resistant species. It is important to correlate these experimental results based on cholesterol-rich diets[36-40] with the observations carried out by De Langen on human subjects. In 1916, he reported that the Javanese were much less prone to develop severe atherosclerotic lesions than the Dutch and this difference was associated with diets; the diet was much lower in fat in Java and the Javanese also showed a lower blood cholesterol level.

B. Where Are We Now?

1. The Significance of the Term "Atherosclerosis"

Introduction in the medical literature of this term is attributed to Marchand. At the Wiesbaden Congress of 1904, he proposed this term to designate a disease characterized by lipid-rich arterial lesions, intimal thickening, fibrosis, and calcification.[41] The presence of lipid deposits was considered a hallmark of atherosclerosis and the main point of differentiation between atherosclerosis and arteriosclerosis. Eminent authorities in the field of vascular pathology have not accepted the term, which literally translated means "gruel-like hardness" and prefer the term "arteriosclerosis". Therefore, papers dealing with the natural history of coronary atherosclerosis can be found under both terms. Each issue of these journals shows that the terms atherosclerosis and arteriosclerosis might mean the same thing for two investigators, or one thing to one investigator and something else to another investigator; these terms are sometimes used to define all types of lesions affecting the artery wall, or, on the contrary, a particular type of lesion. To overcome these difficulties, some scientists used in their reports the combined terms "atheroarteriosclerosis" or "arterioatherosclerosis". In an attempt to clarify some semantic misunderstandings it has been assumed that the Angloamerican term "atherosclerosis" corresponds to "arteriosclerosis" in the German. "Arteriosclerosis" in English apparently sums up atherosclerosis and small vessel disease, which corresponds for German pathologists to the term "arteriolosclerosis" or "hyalinosis".[42]

Many clinicians consider the terms atherosclerosis and arteriosclerosis similar to the terms "coronary heart disease" or "ischemic heart disease". In addition, subjects with severe and diffuse atherosclerotic involvement, but asymptomatic and with a normal ECG, are classified as "healthy" by clinicians. Some angiographers use the term "disease" instead of atherosclerotic plaque. When two or three successive angiographic examinations are made on the same heart and a new stenotic atherosclerotic plaque is discovered, this is recorded as a "new disease".

Ever since Virchow put forward his theory on arterial wall imbibition by plasma components as the cause of atheromas, heated discussion has taken place as to whether the lesions are a prerequisite for, or a result of, plasma imbibition. It is the character of the primary injury to the arterial wall which led to the significant differences in opinions, points of view and definitions of human atherosclerosis. Some selected examples are given here in both chronological and alphabetical order.

Duguid (1946): Atherosclerotic lesions are arterial thrombi which by the ordinary process of organization have been transformed into fibrous thickenings. Red thrombi are prone to softening and fatty degeneration and the atherosclerotic plaques found in arterial thickenings appear to be the result of this process.[43]

Study group of the World Health Organization (1958): Atherosclerosis is a variable combination of changes in the intima of arteries (as distinct from arterioles) consisting of focal accumulation of lipids, complex carbohydrates, blood products, fibrous tissue, and calcium deposits and associated with medial changes.[44]

Burch and Phillips (1960): Atheromatosis or atherosis is a focal proliferation leading to the onset of plaques with large lipid deposits. Arteriosclerosis is conveniently a less binding term and may be related to a pathological process capable of involving the whole arterial tree, characterized by diffuse hypertrophic changes and an increase in fibrous elements of the arterial wall. Although atheromatosis and arteriosclerosis may occur separately, a combination is common.[45]

Crawford (1960): Atherosclerosis is the widely prevalent arterial lesion characterized by patchy thickening of the intima, the thickening comprising accumulation of fat and layers of collagen-like fibers, both being present in widely varying proportions.[46]

Geer, McGill, and Strong (1961): The term atherosclerosis is used to denote an arterial intimal lesion in which fat is present.[47]

Moga and Haragus (1963): Atherosclerosis develops as a result of a long and dynamic interference between an altered metabolism of blood lipids and a modified arterial wall.[48]

Pickering (1963): Atherosclerosis is, in fact, a nodular arteriosclerosis, occurring as fatty nodules, fatty fibronodules, and fibronodules.[49]

Adams (1964): Atherosclerosis is a multifactorial proliferative and degenerative condition that affects the lumen, intima, and the inner part of the media of both large elastic arteries and certain muscular arteries. The proliferative feature of the disease is essentially an organizing or sclerotic reaction of tissues in the tunica intima, while the degenerative element is manifested by lipid accumulation, fragmentation of connective tissue, calcification, and ischemic necrosis of the center of the lesions. Atherosclerotic lesions are both proliferative and degenerative in nature.[50]

Mustard, Murphy, Rowsell, and Downie (1964): Three groups of factors appear to be of importance in the development of atherosclerosis and its complications: (1) the vessel wall, especially the intima and endothelium; (2) the hydraulics of blood flow; and (3) the blood and its constituents.[51]

Page (1966): Atherosclerosis is a multifaceted disease in which the hereditary, conditioned body overreacts to its environment; there is no single cause of atherosclerosis — it is the result of a constellation of factors.[52]

Scott, Daoud, Wortman, Morrison, and Jarmolych (1966): Atherosclerosis can be divided into two phases — a proliferative phase characterized by thickening of the arterial intima and a necrotic phase characterized by focal necrosis.[53]

Friedman and Rosenman (1968): Simple atherosclerosis is a lesion in which a hyperplastic process involves the internal portion of an artery; its beginning is heralded by fragmentation of the internal elastic membrane.[54]

Ooneda (1968): The basic process in the morphogenesis of arteriosclerosis is the combination of proliferation and insudation in the intima. By intima proliferation is meant proliferation of cells and formation of ground substance and fibers by cells, while insudation points to infiltration and stagnation of blood plasma containing lipoproteins. Various types of atherosclerotic lesions are formed by different combination of the two. Atherosclerosis is a focal lesion seen in the large and medium-sized arteries and shows atheroma at the base of the thickened intima and cellulofibrous tissue or fibrosis in the upper layer.[55]

Gore (1971): Atherosclerosis is a patchy, irregularly disposed process involving the intima of large arteries, characterized by focal deposits and elevation of the intima as a consequence of the accumulation of lipids, complex carbohydrates, fibrous tissue, calcium deposits, blood products, and blood.[56]

National Heart, Lung, and Blood Institute (1971): Atherosclerosis in man is characterized in its early stages by extensive accumulation of cell masses in the arterial intima.[57]

Gensini and Kelly (1972): Coronary atherosclerosis is a degenerative arterial disease which in time can progress and produce stenosis or occlusion with reduced myocardial blood flow and result in myocardial damage and death.[58]

Strong, Eggen, Oalmann, Richards, and Tracy (1973): Arteriosclerosis, as properly used, is considered a general term, including all arterial diseases which lead to thickening and hardening of the vessel wall. Atherosclerosis primarily involves the intima of large and medium-sized arteries with characteristic lipid accumulation in the lesions. In addition to the accumulation of lipid, there is an accumulation of connective tissue and various blood products.[59]

Dock (1974): By 1904, when Marchand introduced the term atherosclerosis, the histology of sebaceous cysts was known to be unlike that of arterial lesions. Now we know it is identical with that of xanthomas, and it is high time pathologists, editors, and medical index rejected the errors of the 18th century. We should speak of the intimal lesions as xanthomas and the disease as xanthosclerosis.[60]

Spaet, Gaynor, and Stemerman (1974): Arteriosclerosis and even atherosclerosis may be produced by endothelial injury alone; hypercholesterolemia appears to be necessary, but not essential for significant lipid deposition.[61]

Texon (1974): Atherosclerosis may be considered the reactive biologic response of blood vessels to the effect of the laws of fluid mechanics, namely the forces generated by the flowing blood at sites of predilection determined by local hydraulic specifications in the circulatory system.[62]

Fry (1976): Atherosclerosis occurs at the sites of restructuring of the local conduits' configuration as (1) an error that was not anticipated in the evolutionary design of this self-correcting scheme and (2) an aberration of a physiological response to nonsignificant, reversible, injuries induced as hemodynamic stresses in the endothelial layer.[63]

Gero and Bihari-Varga (1976): The deposition of lipids is not the activator, but only the indicator of pathological connective tissue changes which take place in the vessel wall.[64]

Minick (1976): Injury to the arterial wall is probably a primary causative factor in arteriosclerosis. Arterial injury, the resulting necrosis, inflammation, intimal hyperplasia, and other features of repair may favor the deposition of blood-borne lipid at the site of injury and thereby lead to atherosclerosis.[65]

Benditt (1977): The atherosclerotic plaque may be some form of neoplasma of abnormally proliferating tissue. We frequently tend to confuse the lesions induced in animals with the real human lesions. It seems reasonable to consider the single cell plaque as being monoclonal in origin, and to propose that some event provides a single cell with an advantage over its neighbors and that the progeny of that altered cell dominates the ensuing process of normal replacement multiplication. The commonly accepted reason for the appearance of such a selective advantage in a body cell is an alteration of its genetic apparatus: a mutation. The monoclonal hypothesis holds that the proliferating cells of an atherosclerotic plaque all stem from one mutated cell.[66]

Robertson (1977): Atherosclerosis may be triggered and/or accelerated by the abnormal response of some areas of the vessel wall to the cumulative and often synergistic effects of recurrent injury-repair cycles, resulting in permanent arterial damage.[67]

Stehbens (1977): Hemodynamically induced vibrations affect the etiology of atherosclerosis by inducing mechanical fatigue in the engineering rather than the physiological sense. Lipid accumulation and other degenerative changes could result from associated biochemical disturbances due to vibrational activity of the vessel wall. Hemodynamics is not merely a contributing factor, but the primary cause of atherosclerosis.[68]

Wolinsky and Fowler (1978): Atherosclerosis can be viewed as a complex tissue response by vascular smooth muscle cells, which proliferate, synthesize a broad array of connective tissues, and accumulate lipid.[69]

Constantinides (1979): Atherosclerotic lesions are not merely myocytic proliferation foci. They are highly complex lesions that consist of many and variable components such as increased endothelial permeability; the infiltration of plasma, monocytes, and lipids from blood into the arterial wall; lipid uptake by myocytes and monocytes; myocyte proliferation; the disintegration of lipid-laden cells; the development of extracellular, partly crystalline lipid pools; focal wall necrosis; fibrosis; ulceration; thrombosis; the incorporation of organized thrombi; capillarization and hemorrhage; and other components. Thus, any theory that attempts to explain atherosclerosis must address this whole spectrum of changes, not just one of its components.[70]

Copley (1979): The prevalent change in atherogenesis is the adsorption of low-density lipoprotein (LDL) to the endothelial fibrin lining and/or to the fibrinogen gel thrombus at the site of the developing early atheroma.[71]

Kadish (1979): Atherogenesis represents an abnormal form of intimal injuries exerted by reduced levels of plasma fibrinolytic activity. When considered from this perspective, the

data currently known of risk factors which predispose to atherosclerosis may be viewed as acting through a common central initiating mechanism.[72]

Zilversmit (1979): Atherogenesis is a postprandial phenomenon.[73]

Moore (1981): The presence of lipid in the lesions has been accepted as a *sine qua non* of the atherosclerotic plaque. The merits of this requirement are debatable. The tendencey to equate the presence of lipid in arterial lesions with atherosclerosis tends to make the association between high levels of blood lipid and the occurrence of arterial disease a self-fulfilling prophecy. It is clear that lipid may be present in lesions only at certain stages of their development.[74]

Stehbens (1982): The nomenclature of degenerative vascular disease is currently confusing and needs revision. The term atherosclerosis generally denotes increased fibrous tissue content of the vessel wall in association with lipid accumulation. Though often considered a focal disease of the arterial intima, in reality all layers and all constituents of the vessel wall are involved in certain areas affected earlier and more severely than others.[75]

Woolf (1982): The key expression of the processes concerned in atherogenesis are (1) proliferation of smooth muscle cells within the arterial intima and elaboration of extracellular matrix and (2) accumulation of intra- and extracellular lipid, most of which appear to be derived from the plasma.[76]

Baroldi (1983): Atherosclerosis seems a pathologic process specific for a certain stage in the evolution of the human race. From the morphologic standpoint, atherosclerosis can be defined as a progressive thesaurismotic-degenerative process, alternating with reactive fibrosing repsonses leading to a reduction of the vessel lumen. In contrast, arteriosclerosis, particularly in the coronary arteries, means a primary myoelastic intimal proliferation progressively substituted by fibrous tissue with loss of elasticity and medial atrophy, resulting in lumen dilatation or ectasia.[77]

Benditt, Barrett, and McDougall (1983): There are several ways in which viruses could operate in the pathogenesis of atherosclerosis; they could induce proliferation of artery wall intimal smooth muscle cells via injury or by genetic alterations leading to clonal expansion of intimal smooth muscle cell populations.[78]

Gresham (1983): It is reasonable to regard atherosclerosis as a proliferative response to vascular injury and all later changes such as accumulation of lipid, fibrin, etc., as a secondary event.[79]

Mahley (1983): Atherosclerosis can be defined as a disease process that occurs when the influx and deposition of cholesterol into the arterial wall exceed the egress of cholesterol from the arterial wall.[80]

Ross (1983): Atherosclerosis is a question of endothelial integrity and growth control of smooth muscle cells. There is reasonably widespread agreement that the intimal smooth muscle proliferative response is a key event if lesions of atherosclerosis of clinical consequences are to occur.[81]

Schettler (1983): The development of atherosclerosis is determined by two pathogenetic factors: the arterial wall and the contents of the vessel. Both factors should be considered as pathogenetic equals.[82]

Strong (1983): Atherosclerosis is a specific form of arteriosclerosis. The most distinctive feature of atherosclerosis is the accumulation of lipid in the intima of large elastic arteries and medium-sized muscular arteries. In addition to lipid, cells, connective tissue fibers, and various blood products accumulate in the lesions. The hallmarks of atherosclerosis are its intimal location and the accumulation of lipid. Atherosclerosis is the form of arteriosclerosis that most frequently causes clinically significant disease.[83]

Thomas and Kim (1983): Atherosclerosis is primarily a proliferative disease of the arterial intima with the accumulation of necrotic, lipid-rich calcific debris seen in advanced stages as a secondary feature; the proliferation probably represents a hyperplastic response to injury,

rather than neoplasia. However, the possibility of the development of occasional neoplastic foci as a secondary feature cannot be entirely eliminated.[84]

Engelberg (1984): Human atherogenesis involves the reaction of the endothelial and smooth muscle cells of the arterial wall, the action of platelets and their products, factors contributing to abnormally elevated plasma lipid levels, and aberrations of coagulation that predispose to thrombus formation. Hemodynamic forces also play a role in the localization and acceleration of atherosclerotic lesions.[85]

Kuller (1984): Coronary atherosclerosis is an example of the continuous exposure type to a common epidemic source; in this case, it is most likely saturated fat and cholesterol in the diet. The population at risk of the disease is a function of the extent of exposure to the agent, i.e., the amount of saturated fat in the average diet of the population.[86]

Motulsky (1984): Coronary atherosclerosis often represents the end results of a genetically determined differential response to various environmental factors.[87]

Ross, Wight, Strandness, and Thiele (1984): Contrary to earlier beliefs, atherosclerosis is not initially a degenerative process. Instead, the lesions of atherosclerosis are proliferative, particularly during their development and progression. The degenerative changes so commonly observed are a manifestation principally of changes that occur in relatively advanced lesions.[88]

Strasser (1984): Coronary atherosclerosis is not a direct consequence of affluence, but a sequel of some of its unwanted behavioral side effects, such as unbalanced nutrition and smoking.[89]

Wissler (1984): It is now evident that atherosclerosis is a dynamic process in which a number of stimuli to arterial lipid deposition, cell proliferation and cell death, and the synthesis of fiber and matrix proteins by modulated smooth muscle cells are the major factors which can vary to control the process. Most of these functional components appear in turn to be under the direct influence of the circulating levels and interaction of lipoproteins, the integrity of the endothelium, the heart rate, and the height of the blood pressure and many other arterial wall factors not yet clearly understood.[90]

Jerome and Lewis (1985): Atherosclerosis is a focal disease of the arterial intima. The earliest stage of the disease involves proliferation of vascular smooth muscle cells, infiltration of blood cells, and the accumulation of lipid. Because of the focal nature of the disease, it is presumed that the early factors in the pathogenesis of atherosclerosis include local alterations in the arterial wall which promote the accumulation of blood lipids and inhibit intramural lipid clearance.[91]

Kádár and Björkerud (1985): The main feature of an atherosclerotic plaque is the formation of a new tissue in the arteries. In this respect, atherosclerosis is similar to other conditions where non-neoplastic tissue formation occurs as in embryogenesis, in healing, or in repair processes. The behavior of smooth muscle cells is similar in human atherosclerosis to experimental conditions where any sort of injury causes endothelial damage which is followed by smooth muscle cell proliferation and the development of scar tissue.[92]

Kannel (1985): There is a growing conviction that atherosclerotic cardiovascular disease is chiefly a product of a faulty lifestyle or adverse environmental influences, but only cigarette habit, dyslipidemia, and hypertension are clearly causative.[93]

Lindner (1985): Atherosclerosis is an arterial connective tissue disease which is first demonstrable by the increased glycosaminoglycan metabolism and smooth muscle cell turnover rate. These are correlated as primary processes to the secondary disturbed metabolism of collagen, elastin, lipid, and coagulation factors.[94]

Rank (1985): The term atherosclerosis has unduly stressed the role of lipids resulting in an inappropriate conceptual emphasis; rather, arteriosclerosis is the preferred term. Use of this term underscores the fact that arteriosclerotic lesions routinely contain connective tissue and other cellular elements as principal components, that lipid is often not a major feature of the disease, and that the disease frequently exists without lipid deposition at all.[95]

Schwartz, Spague, Kelley, Valente, and Suenram (1985): Along with Joris and Majno,[97] we consider that atherosclerosis fulfills the contemporary criteria defining an inflammatory process in which the peripheral blood monocyte is an important participant. One role is its scavanger or phagocytic function, and another progenitor of at least part of the macrophage-foam cell population of the plaque.[96]

Schwartz and Heimark (1985): The onset of atherosclerosis involves two changes: a morphogenetic change required to remove the cells from their normal environment, permitting migration, as well as proliferation and a proliferative state that might represent either the response to exogenous growth factors or synthesis of endogenous factors by cells released from normal control. Development of a lesion requires that smooth muscle cells migrate from the media into the intima and grow in the quite different environment of the intima.[98]

Smith (1985): It is really the lesion which determines the amount of LDL rather than the LDL that creates the lesion. The role of LDL is secondary; it does not initiate lesions, but causes their progression.[99]

Wiklund, Carew, and Steinberg (1985): The main characteristics of the developing atherosclerotic lesion are cell proliferation and increase in extracellular matrix and cholesterol accumulation.[100]

Hough and Zilversmit (1986): Accumulation of cholesteryl ester is the primary feature of the atherosclerotic lesion.[101]

Ross (1986): The lesions of atherosclerosis are proliferative smooth muscle lesions which may result from various forms of endothelial injury or dysfunction, associated with the different risk factors for atherosclerosis. These lesions have been associated with interactions between platelets and macrophages, and with substances released from these cells, including growth factors. The lesions may represent defensive responses that have not subsided, but instead progress to a pathologic response.[102]

In our material, coronary atherosclerosis appeared as a complex proliferative, necrotizing, and accumulative arteriopathy involving the intima of the major coronary arteries and of their main branch vessels, as well as the intima of the veins grafted in the coronary arterial trees. It gave rise to stenotic fibromuscular, fibrohyaline, and lipid-rich fibronecrotic plaques and to mural and occlusive thrombi. We have revealed in our material many lipid-free atherosclerotic plaques, gelatinous lesions, and intimal necrotic areas. Lipid-free atherosclerotic lesions were also detected by many pathologists in the arteries of Oriental, African, and South American populations, in the arteries of wild, captive and domesticated animals with spontaneous atherosclerosis, and in the aortocoronary bypass grafted veins. A lipid-free plaque may also result following regression diets and/or drug treatments and it remains an atherosclerotic lesion, in spite of the disappearance of its lipid content. Recent wars were followed by a substantial decline of lipid-rich lesions in the coronary arteries of subjects submitted to a reduced fat intake; this aspect can be encountered in the final stage of some cancers and of liver cirrhosis. On the other hand, lipid-rich accumulations can be visualized in placental "atherosis", in aging tendons and fascia, the synovial membrane, lymph nodes, many granulomatous tissues, necrotic areas detectable in tuberculosis, sarcoidosis, and Hodgkin's disease. In essence, we do not consider the presence of sudanophilia necessary in order to detect or delineate an atherosclerotic lesion. Moreover, we disagree with investigators who use the terms "hyperlipoproteinemia" or "hyperlipidemia" as similar with the term atherosclerosis. This use is scientifically inaccurate, since hyperlipidemia is only a laboratory finding and may be a manifestation of many diseases which have nothing to do with coronary atherosclerosis.

To sum up, as there is no unanimity concerning the definition of atherosclerosis, at present each writer should make clear from the onset in his work what this term implies. This absence of an adequate definition is overlooked in many textbooks, monographs, book chapters, and reviews. There is also no evidence that the value of innumerable meetings on

atherosclerosis was in any way diminished by the absence of an adequate definition of the disease on which the discussions were focused. On the other hand, the faminly practitioners, as well as may pathologists, experimentalists, biochemists, and angiographers, seem to have little or no problem in understanding what is meant by the term "atherosclerosis".

Neither was the absence of an adequate definition of atherosclerosis an impediment for many physicians to move from a position of passive observation of the effects of risk factors for coronary heart disease to an active role in identifying heavy smokers and hypertensive and hyperlipidemic patients, in an effort to put into practice that which is now feasible: control of these risk factors, so that certain improvements in morbidity and mortality related to myocardial infarction and sudden cardiac death can be obtained.

This empirical approach is, in fact, the only one available. It is in agreement with the lack of an adequate definition of the disease, of a sound experimental basis, and of adequate etiopathogenetic data to approach the natural history of coronary atherosclerosis.

2. The Significance of Other Terms Used

The myocardial clinical manifestations which occur as end stages in the natural history of coronary atherosclerosis are included in this book under the generic term "coronary heart disease". In other works, the terms "ischemic heart diesase" or "atherosclerotic heart disease" are used in a similar sense. All these terms refer to a *myocardial impairment due to an imbalance between the coronary blood supply and myocardial demand caused by changes in the coronary circulation.*[103] This imbalance may lead to various forms of angina pectoris or myocardial infarction and to severe arrhythmias followed by sudden cardiac death. In this book we have mainly used the term "coronary heart disease" to emphasize that the myocardial damage is produced by coronary lesion; this myocardial damage may also be produced by coronary vasospasm, intracoronary emboli or platelet aggregates, coronary steal, excessive myocardial hypertrophy, and many other mechanisms which induce myocardial ischemia. Adequate knowledge of these mechanisms also requires a clear understanding of the significance of a new term, "cardioneuropathy".[104] This term refers to all abnormalities of the cardiac nerves and ganglia related to sudden cardiac death and other myocardial clinical manifestations.

In the medical literature an unfortunate tendency to use the terms coronary heart disease, ischemic heart disease, or atherosclerotic heart disease as synonymous to coronary thrombosis, coronary occlusion, myocardial ischemia, degenerative heart disease, coronary or myocardial insufficiency, etc., exists. An unfortunate tendency also exists to consider the risk factors for coronary heart disease similar to the risk factors involved in atherogenesis and to present coronary atherosclerosis as the unique possible cause of myocardial clinical manifestations. In line with this concept progression to advanced atherosclerotic lesions is presented similar to progression to clinical disease, overlooking the existence of silent myocardial ischemia and of noncoronarogenic myocardial injuries. Additional erroneous assumptions which persist are that sudden cardiac death is only a particular clinical manifestation of a myocardial infarction and that coronary heart disease is a single clinicopathologic entity.

The terms "thrombosis" and "thrombus" are often substituted for one another in papers dealing with the thrombogenic theory of atherosclerosis. These terms have different meanings: (1) "thrombosis" signifies a pathological condition occurring in the coronary arteries which may lead to myocardial infarction or sudden cardiac death; (2) "thrombus" is an asymptomatic blood clot formed in the coronary vessels, sometimes nonrelated to coronary heart disease. Many small coronary thrombi develop during childhood, adolescence, and adulthood without important pathologic changes. Only few of these thrombi may give rise to thrombosis able to obstruct the vessel lumen.

Likewise, the term occlusion is variously used to describe atherosclerotic plaques with or

without superimposed thrombi, that apparently reduce over 50, 75, or 95% of the luminal diameter, or produce complete obstruction (100% luminal insufficiency).

The term "ischemia" is very loosely used in the available literature. The great majority of investigators consider that it is possible for ischemia to appear only if a circulatory system is present and operational; there are also investigators who employ this term to describe in vitro experiments in which unperfused excised tissue is submitted to autolysis. Ischemia has recently been defined as a reduction in blood flow such that the oxygen supply is insufficient to provide enough energy from oxidative phosphorylation to meet the metabolic demands of the cells, hypoxia being considered an important element of ischemia.[105] Ischemia, the most frequent change during the end stages of the natural history of coronary atherosclerosis, is presented in this book as the result of an imbalance between myocardial demand for, and the vascular supply of, coronary blood. Anoxia and hypoxia are different from ischemia and the terms must not be used as synonymous. In anoxia and hypoxia, the oxygen delivery to the myocardium is insufficient to provide enough ATP by oxidative phosphorylation to meet with energy expenditure, while coronary flow is maintained or even elevated and substrate delivery and metabolite removal may be normal. Ischemia can be associated to tissue hypoxia due to insufficient arterial flow and the presence of anaerobic glycolysis as the dominant energy generating pathway. However, both anoxia and hypoxia differ from ischemia due to the persistence of coronary perfusion, washout of metabolites, and entry of substrates present in coronary blood flow. Ischemia leads to the accumulation of metabolic products that further modify the rates of biochemical reactions. This is usually followed by the cessation of contractions, associated with alterations of the membrane potential and a shift form aerobic to anaerobic metabolism. Anaerobic glycolysis and the accompanying accumulation of glycolytic intermediates, especially lactate, is an invariable metabolic marker of the onset of acute ischemia.[106] Silent myocardial ischemia can be defined as a transient alteration in myocardial perfusion, function, and electrical activity in the absence of chest pain or the usual anginal equivalent.[107] Some confusion may result from an inadequate use of the terms "jeopardized" and "condemned" myocardium. These terms are not synonymous, since in jeopardized tissue, cell injury is considered reversible, because the degree of ischemia is not severe. These cells receive a suboptimal flow and although their capacity to contract may be severely impaired, they are able to remain viable. On the other hand, a condemned tissue is irreversibly injured by severe and prolonged ischemia and its salvage is not possible.

In this book we very frequently use the term "accidental death", which refers to apparently healthy people dying a violent death before receiving medical assistance, without resuscitation attempts, without any record of previous significant diseases. Middle-aged individuals of this group presented severe coronary atherosclerosis and focal area of myocardial fibrosis, both asymptomatic and compatible with a "normal" lifestyle.

The term "stress" is used very loosely in many works; it means stimulus to some investigators, response, interaction, or complex combination of these to others. The term "hemodynamic stress" is limited to any of the identifiable mechanical forces which are exerted per unit wall area by the blood pressure and/or flow. As for "psychosocial stress", it incorporates all the problems with which an individual may be burdened, which demands exceeding his potential ability for adaptation. Particular attention is centered on the psychosocial stress in subjects with "type A" behavioral patterns, attributed to an individual exhibiting excessive drive, impatience, and suffering from a sense of time urgency.[108,109] Psychosocial stress usually aggravates hemodynamic stress.

Much (unexpected) significance has been placed on the term "precursor". In many epidemiological studies, particularly those carried out on children, this term is used as synonymous to risk factors, arterial hypertension being considered by some investigators a risk factor of atherosclerosis, by other investigators a precursor of atherosclerosis; as for

labile hypertension it is presented as a precursor of both sustained hypertension and coronary atherosclerosis. The term precursor of atherosclerosis is attributed to body habitus, body weight, body mass index, various anthropometric measurements, the assessment of sexual maturity, the fat composition of human milk, triglycerides of the diet, etc. The early lesions, particularly those of the fatty streak type are also described as precursors of more advanced plaques, whereas the advanced plaques are considered to act as precursors of occlusive thrombi. This tendency to take all things in a lump generates confusion.

Many investigators use the term "sudanophilic lesion" as equivalent of both human and experimental atherosclerosis. The material presented in this book shows that only a portion of the atherosclerotic lesions is sudanophilic and therefore it is impossible to obtain adequate quantitative data based on sudanophilia alone. An apparently sudanophilic lesion may represent, in fact, an overloaded physiologic clearance system based on the scavenger fucntion of monocyte macrophages; a sudanophilic lesion may also represent a pure fatty streak rich in lipid-filled smooth muscle cells, as well as an unraised foam cell-rich or a necrotic plaque with interstitial lipid deposits, a fibronecrotic plaque with a fibrous cap rich in lipid-filled cells, or a fatty metamorphosis of an intramural thrombus. If the term "normal" commonly carries the implication of the usual or expected, is the presence of sudanophilic lesions in the aortic valve region of all children a usual, expected, or pathological change? Since all children show sudanophilic lesions in their aorta, even those that died during postwar famine, this means that the term normal cannot be used for this vessel by all who consider fatty streaks as pathological changes. There are also collections of lipid-filled cells in the coronary arteries of full-term fetuses. If these cell collections are considered lesions, the number of neonates and infants with normal coronary arteries appears negligible. Also related to the use of the term normal is the tempting question of whether coronary intimal thickening is a normal phenomenon, as presented by many investigators, irrespective of the degree of intimal thickness and intimal microarchitecture. Is the increase in intimal thickness with age a normal or a pathological phenomenon and if it is a pathological phenomenon at what point must we use the term "pathological" instead of "normal"? Without a clear and proper understanding of normal it is a very difficult task to explore the abnormal in relation to the natural history of coronary atherosclerosis. The difficulties appear at all moments in choosing normal cutoff values for use in clinical and public health practice. There are also many difficulties in comparing patients with myocardial clinical manifestations to normal subjects or controls of the same age, sex, and race. In fact some "controls" may have more severe atherosclerotic involvement in their coronary arteries than the patients with myocardial clinical manifestations. The conventional way of defining normal values in medicine is to make measurements in a large population of individuals and to determine the mean and standard deviation. Any value within two standard deviations of the mean is considered to be "normal". This is only theoretical, since there is practically no clear consensus on what should be regarded as normal, one of the most demonstrative examples being blood cholesterol and blood pressure levels. What is normal at any particular age in men and in women, in white and black, in England or China, in subjects with or without the common type of distribution of the coronary arteries? A regrettable confusion exists between the terms normal, lesion, and disease. Particularly angiographers visualize lesions occurring as stenotic atherosclerotic plaques and record this change as a "disease". It is customary for the angiographer to describe a focal atherosclerotic plaque only in terms of the percent luminal diameter or area narrowing it produces and to call it "disease". The most vexing part of this inaccuracy is that we currently use the term disease in a totally different way. Moreover, coronary atherosclerosis is a disease without its own clinical manifestations; when they appear, these clinical manifestations belong to the myocardium and not to the "disease" recorded on coronary angiography. It is also customary for the angiographer to report adults with "normal" coronary arteries, whereas on post-mortem examination all adults are involved by

atherosclerotic lesions in their coronary arteries, the difference standing only in the severity of this involvement. Therefore, the use of the term "normal" is inadequate in such circumstances, since an atherosclerotic lesion is not present only when it becomes visible angiographically. In our opinion it is also improper to record as "normal" all intimal surfaces of the major coronary arteries which are not covered by fatty streaks and raised lesions on post-mortem examination. This inevitably leads to the incredible assumption that in mature adults and elderly people more than 50 or even 75% of the major coronary arteries are normal. Successive longitudinal sections made from these vessels usually show that this "normal intima" does not exist and that the entire length of the proximal segment may be diffusely involved by atherosclerosis.

The loose and perhaps incorrect use by epidemiologists of the term "cause" may create confusion and may inhibit interdisciplinary communication and understanding, as demonstrated in a recent work.[110] In certain papers a factor which represents the sole prerequisite in the production of a disease is considered similar to associated factors which play only an episodic role. The extended epidemiological use of the word cause to encompass contributing, modifying, predisposing, and conditional factors is open to discussion. As concerns coronary atherosclerosis, we are still unable to offer adequate data on the specific agents that must be present before the lesion can develop; in agreement with other investigators we can only suggest that *environmental conditions are associated with the real cause without being directly causative.*[111] It is scientifically accurate to differentiate between cause and noncausative factors, since *elimination of the cause eradicates the disease,* whereas *elimination of the noncausative factors only ameliorates or reduces the incidence of the disease.*

II. SOME PARTICULAR FEATURES OF HUMAN CORONARY ARTERIES

A. Coronary Circulation

1. General Observations

The primary function of coronary circulation is to supply the metabolic needs of the heart, an unusually close link existing between myocardial perfusion and metabolism.

Because the heart is a constantly working muscle that may speed up or slow down from one moment to another, its blood flow must be constantly regulated, the facility with which blood passes through the coronary arterial bed being critical for the performance of the heart. In this respect, the coronary arterial bed is equipped with many control systems that serve this regulatory function, allowing each vascular segment to adapt the distribution of flow to the events of the cardiac cycle and to the myocardial metabolic rate. It is this strong relationship between each vascular segment of the coronary arterial tree and the myocardial region supplied by the respective vessel which explains total myocardial dependence upon the functional and structural integrity of the coronary arteries.

Some investigators consider it appropriate to present the coronary arterial bed as being composed of conductive and resistive segments, controlled by different physiologic stimuli and mechanisms.[112] According to this concept, large and small coronary vessels would respond differently to the same stimuli; sympathetic neurohumoral stimulation of adrenergic receptor activity appears to be particularly strongly influenced by the size of the vessel. In certain studies, vasoconstriction of large coronary vessels and dilation of small coronary vessels were the primary actions of epinephrine and norepinephrine; in other studies, an increase in coronary vascular resistance could be recorded in response to either cardiac sympathetic nerve stimulation or to the administration of sympathomimetic amines in both large and small vessels. The large vessel response could be abolished after α-adrenergic blockade, whereas the small vessel was usually masked by the concomitant vasodilator action accompanying adrenergic stimulation.[112] The particular distribution of α- and β- receptors in large and small coronary vessels was also recorded, and this may be related to the

differences detected in the response to various stimuli. The ratio of α- to β- receptor activity was found to be augmented in the coronary arterial bed with increasing vessel diameter. This would indicate that small coronary vessels possess more β- than α-adrenergic receptors; on the other hand, large coronary arteries appear to possess more α-adrenergic receptors than β.

An important role in the response of various segments of the coronary arterial bed to stimuli is played by the mechanism of *autoregulation* (the coronary circulation is considered an autoregulated vascular bed).[113] An additional particular feature is that in coronary medial smooth muscle cells, contraction seems to be maintained in the presence of a low free calcium concentration.[114]

The tension produced by blood pressure in each segment of the coronary arterial tree appears to be related to vessel diameter. To estimate and compare this tension and thereby determine the increase in functional load from one segment to the other, a simple law of mechanics is usually applied, the law of Laplace. It states that the total force or tension (T) in the vessel wall represents the product of the radius of the vessel (r) and the blood pressure (p): T = r.p. According to this law, tension increases in the coronary arterial bed with the radius of the vessel and with blood pressure, the highest being recorded in the major coronary arteries: left main trunk, anterior descending, circumflex, and right coronary arteries.

The coronary arterial tree is considered to have the "dubious distinction" of aging more rapidly and becoming obstructed by atherosclerotic plaques more commonly than any other arterial system.[115] Contrary to data furnished by certain in vitro models, the coronary arterial bed does not consist of rigid circular pipes, with a constant rate and velocity profiles; it is formed by vessels with a very complex and variable microarchitecture and with very complicated fluid dynamics. The unsteadiness of the flow and additional phenomena such as turbulence, secondary flows, flow separation and other changes in the flow pattern produced by entrance effects associated with abnormal shear stresses related to excessive branching, vessel curvature, and rapid tapering (all peculiar to the coronary arteries) must be taken into consideration. The coronary arterial tree is also subject to torsion, spiral twisting, and bending with every myocardial contraction, and these also contribute to the particular flow patterns of these vessels.

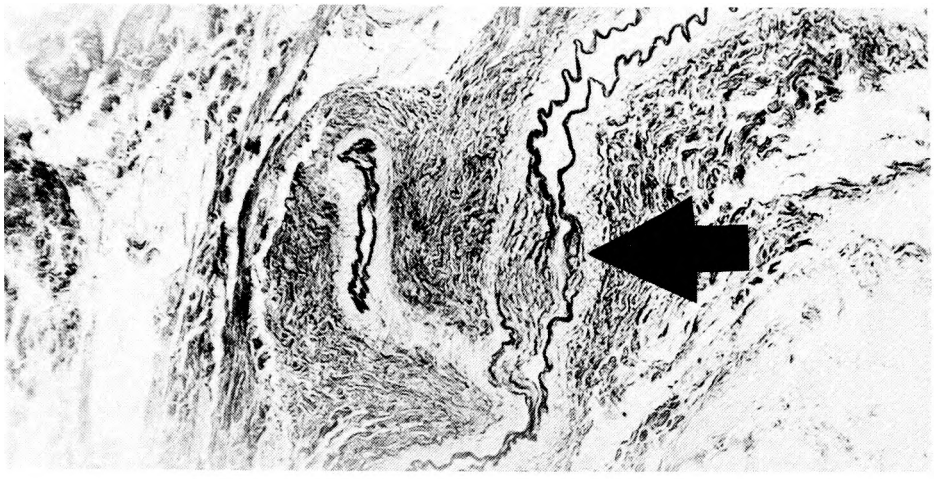
In considering these peculiar hemodynamic stresses, some ontogenetic changes that appear in the coronary circulation must be borne in mind. The primitive heart is nourished only through lacunar or intertrabecular spaces which communicate directly with the heart cavities. The coronary arteries are formed during the 7th week of intrauterine life, serial sections being necessary to reveal their occurrence in embryos having a crown to rump length of 14 to 15 mm. These vessels encircle the heart along the inter- and atrioventricular grooves and their first ramifications penetrate the myocardium joining the lacunar space.[77] During age-related growth of both ventricles these lacunar spaces are progressively reduced and assume the pattern of the terminal arteriolar, pericapillary, and capillary bed of the coronary arterial tree. A dramatic change also occurs during prenatal life and continues after birth; it includes the passage from right ventricle preponderance and a pulmonary pressure higher than the aortic, toward left ventricular preponderance and an aortic pressure higher than the pulmonary. These changes develop in such a way that the pumping load remains balanced despite the different prevalences. On the other hand, a reduction in the shunt from the pulmonary circuit to the aorta (disappearance of the ductus arteriosus as a functional conduit) associated with the newly assumed respiratory activity of the lung may influence the hemodynamic pattern of the coronary arterial bed. It has now been clearly demonstrated that after birth, with the structural closure of the ductus arteriosus, the left ventricle must carry the full load of the systemic circulation, with no assistance from the right ventricle. This is followed by a progressive increase in the thickness of the left ventricle and in the amount of its blood supply. The passage from "water to air-living" leads to a considerable development of the myocardial areas supplied by the left anterior descending and left circumflex

arteries. The role of these two vessels is considered unique because they perfuse the left ventricle which generates the perfusion pressure for the entire circulation.[116] The terminal branches with the surrounding myocardial cells seem to form "functional elements" of the heart and represent a particular form of microarchitecture interposed between cells and the whole organ.[117] These microcirculatory units may be separately influenced by the nervous system and catecholamines. Their structural organization was revealed to be different in the epicardial and endocardial areas. Also, as a result of the passage from water to air living and the considerable postnatal development of the left ventricle, the major coronary arteries carry different volumes of blood to this ventricle. The left main coronary artery (left main trunk) carries to the left ventricle approximately five times the amount of blood than the right coronary artery. The left circumflex artery carries 1.5 times and the left anterior descending artery 3.5 times more blood than the right coronary artery.[118] Roentgenograms of transverse serial sections of the ventricular myocardium enable planimetrical measurements which revealed that 41.5% of the entire ventricular myocardium is supplied by the left anterior descending artery. Together with the left circumflex artery, both vessels supply an average of 63.8% and the right coronary artery supplies 36.2% to the myocardium.[118] This significant difference in the volumes of blood carried by the major coronary arteries to the myocardium and, particularly, to the left ventricle could account for the different pathophysiologic consequences in the presence of stenotic atherosclerotic plaques of similar severity and similarly bypassed by collateral vessels. There might also be noticeable differences in the autonomic neural control or even differences in vasomotor responses between the left anterior descending and left circumflex arteries, on the one hand, and the right coronary artery, on the other. In the dog at rest, the systolic-diastolic coronary flow ratio appeared to be significantly greater in the right coronary artery than in both the left anterior descending and left circumflex arteries. This may reflect less reserve capacity in the right than in the left vessels and all these results demonstrate that the presentation of the coronary arterial bed as an anatomical and functional entity is a very difficult task.[119]

An additional important ontogenetic change which could influence coronary blood flow and myocardial blood supply is related to the development of branch pads or cushions. These genetically determined structures are commonly present in the human coronary arterial tree and have been described by many investigators, but their real functional significance is largely unknown. Many physiologists and experimentalists overlook the existence of branch pads or cushions in the coronary arteries, or assume, in a general manner, that they are a normal feature of coronary vessel microarchitecture which permit the adaptation to circumferential tension and additional hemodynamic stresses at the sites of branching.

Branch pads or cushions which encircle the orifice of the branch, occurring as ring-like structures that protrude in the lumen, were seen in our material as early as in the 5-month-old fetuses, at the bifurcation area of the left main coronary artery (Figure 1). In 6-month-old fetuses we could detect branch pads or cushions in the paraostial region of both left anterior descending and left circumflex arteries (Figure 2). During the 5th and 6th months of fetal life, pads also developed in the aortic branch sites of the left main and right coronary arteries. Finally, during the 8th and 9th months of fetal life, cushion-like thickenings were visualized in the main branch sites of the coronary arterial tree and by means of serially cut sections even at the branch site of the vessels supplying the conduction system (Figure 3). In the coronary arterial tree of children, branch pads or cushions were often seen in vessels of differing size (Figure 4). Some of these pads encroached upon the vessel lumen and significantly reduced the entrance region of the branch area and the diameter of the branch mouth (Figures 5 and 6).

As compared to other similar structures of the coronary arterial tree, the pads of the branching zone of the proximal segment of the left anterior descending artery exhibited the more complex histologic feature, the greatest development of longitudinally arranged bundles



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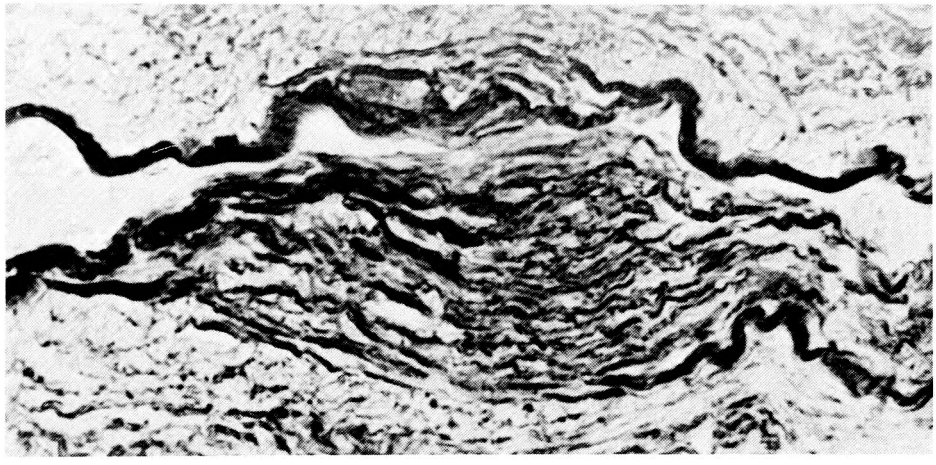


B

FIGURE 1. (A) The first branch pad or cushion detected during the ontogenetic evolution of the coronary arterial tree. It appeared in our material in 5-month-old fetuses, in the bifurcation region of the left main coronary artery (arrow). (B) The light microscopic feature of this first branch pad or cushion, showing fragmentation of the internal elastic membrane and abundant new formation of elastic fibers. (Resorcin fuchsin-alcian blue. Magnification (A) × 70, (B) × 440.)

of smooth muscle cells, the greatest thickness, and the greatest tendency to extend outside the branch site. As compared to other muscular arteries of similar size, the branch pads or cushions of the coronary arteries also showed some particular features. In a comparative study on the branch pads of the coronary, basilar, and renal arteries, pads appeared as intimal structures and exhibited a precocious alteration of the underlying elastic membrane only in the coronary arterial tree. In the basilar and renal arteries, pads developed only in the media under the internal elastic membrane or between its reduplicated laminae, without overlying intimal connective tissue (Figure 7).[120] The significance of these differences remains speculative, but the role played by genetic and local hemodynamic factors cannot be underestimated.

Four decades ago, it was shown that the size of the coronary arteries varies directly with the heart weight in both normal and hypertrophic myocardium,[121] and is directly proportional

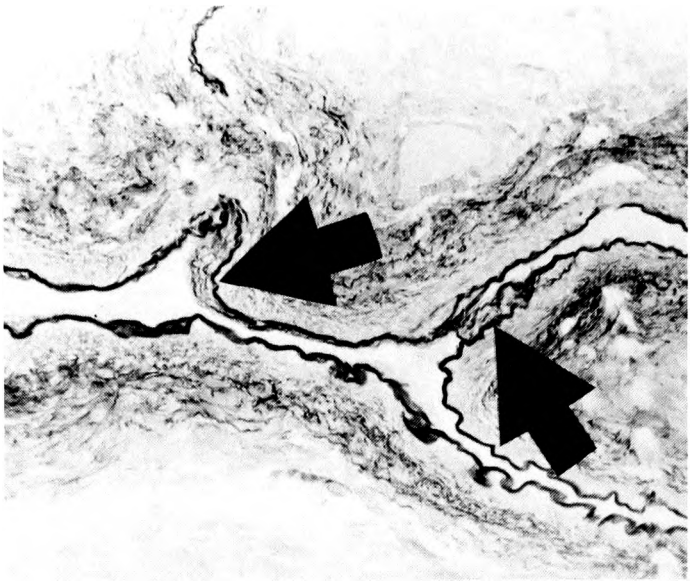


FIGURE 2. Development of branch pads or cushions (arrows) in the entrance region and paraostial area of the left circumflex and left anterior descending arteries of a 6-month-old fetus. (Resorcin fuchsin-alcian blue. Magnification × 70.)

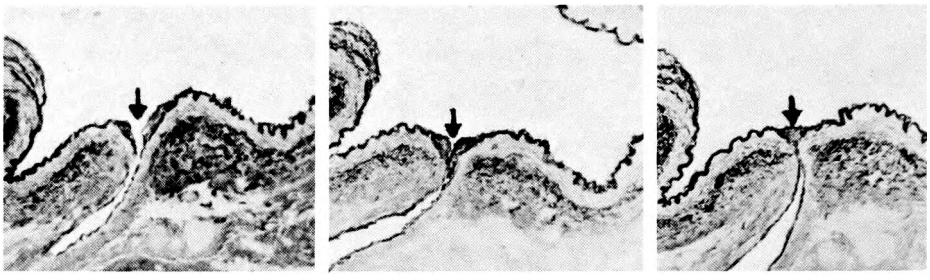


FIGURE 3. Full-term fetus. Serial sections showing the presence of a well-developed branch pad or cushion at the branch site (arrow) of the right coronary artery which gives rise to the sinus node vessel. (Resorcin fuchsin-alcian blue. Magnification × 120.)

to the heart weight. This would indicate that *the blood flow volume required by the contracting heart determines the functional capacity of the coronary arterial bed.* A complex mechanism seems to exist which is able to regulate the muscular tone of major coronary arteries, main branch vessels, small arteries, and arterioles, in a manner that provides maximum economy of energy expenditure. When this mechanism acts efficiently, the coronary blood flow increases in proportion to the demands of the contracting myocardium. Capillaries also run parallel to the contracting myocardial cell and by this arrangement minimize the distance for oxygen diffusion; in addition, there are anatomical peculiarities which might permit arteriovenous diffusional shunting. This diffusional shunting may be important in the delivery of oxygen to, or removal of, metabolites from the environment of each myocardial cell.[122]

A peculiar diffusional shunting which could play an important role particularly in the end stages of the natural history of coronary atherosclerosis is *coronary steal.* Pressure changes in the coronary circulation at the origin of collateral vessels usually leads to coronary steal; namely an increase in flow to nonischemic myocardium at the expense of decreasing collateral flow to ischemic myocardium at constant aortic pressure, perfusion rate, and heart rate.

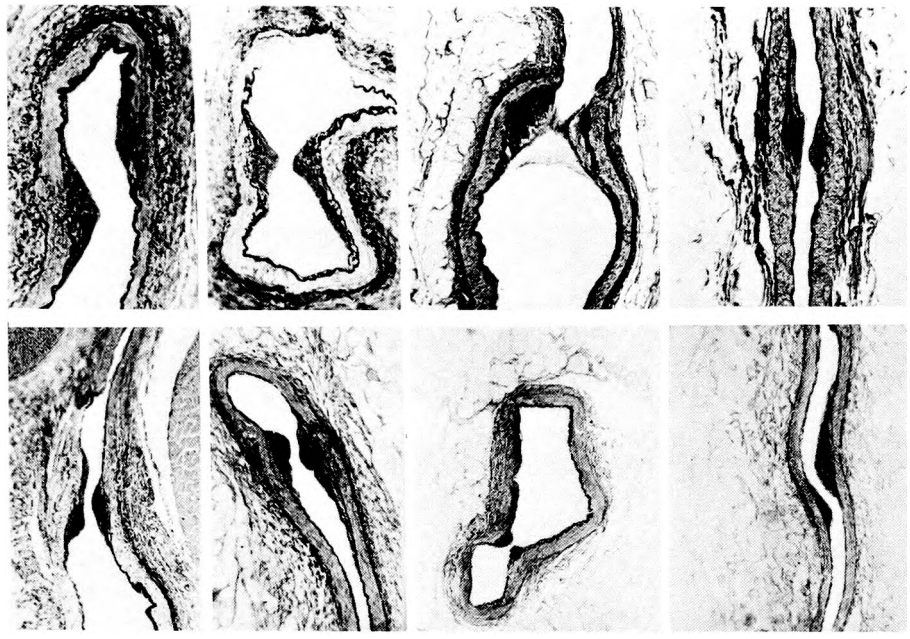


FIGURE 4. The presence of branch pads or cushions is a frequent light microscopic finding in the coronary arteries of children aged 1 to 10 years. (Resorcin fuchsin-alcian blue. Magnification × 70.)

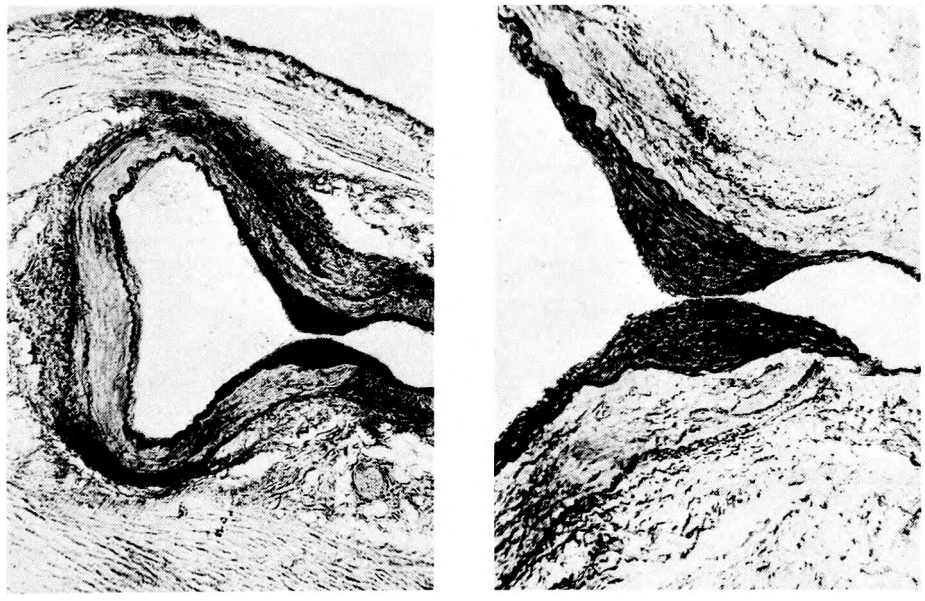


FIGURE 5. Branch site of the left circumflex artery of a 3-year-old male child and the branch mouth of the left marginal branch. Important development of the branch pad or cushion which encroaches significantly upon the branch mouth lumen. This important luminal narrowing persisted in serially cut sections. (Resorcin fuchsin-alcian blue. Magnification, left × 220; right × 440.)

Conflicting evidence exists regarding whether coronary steal develops when a major coronary artery is occluded and the others are partially narrowed, or whether a major coronary artery is occluded and the others are patent. It is reasonable to believe that when changes in branch pads or cushions lead to the occlusion of the branch mouth of collateral vessels, an increase in the magnitude of coronary steal is to be expected in proportion to the decrease in coronary

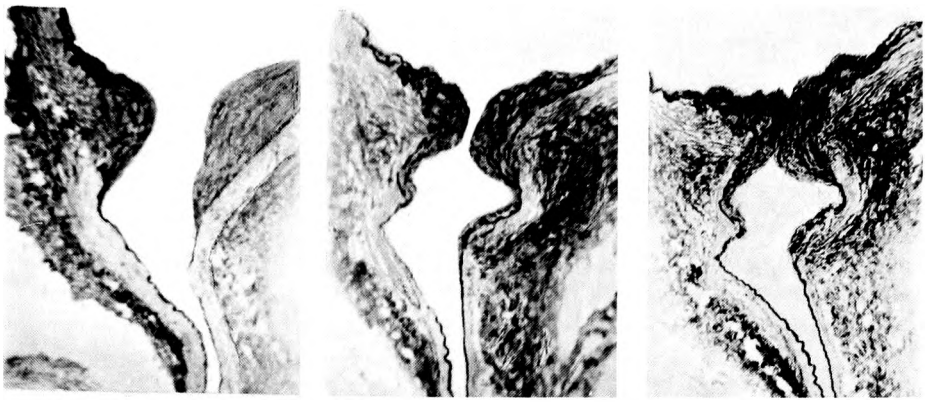
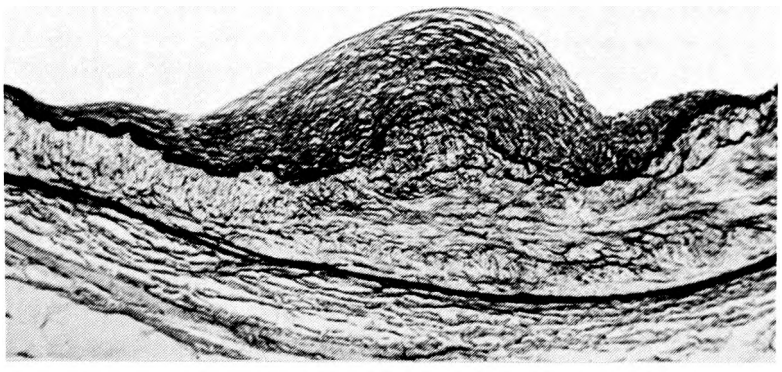


FIGURE 6. Semiserially cut sections at the branch mouth of the first diagonal vessel of a 7-year-old male child. The largest diameter detected in present in the first (left) micrograph. The successive aspects showed that branch pads or cushions encircled the orifice of the branch, occurring as ring-like structures. (Resorcin fuchsin-alcian blue. Magnification × 220.)



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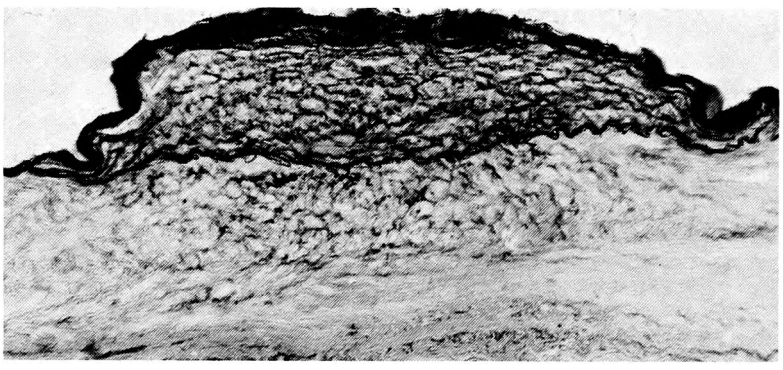


B

FIGURE 7. Comparative aspects of the microarchitecture of the pads of the coronary left anterior descending artery (A) and basilar artery (B) of a 5-year-old male child. The pad of the major coronary artery is an intimal structure, with underlying interruption of the internal elastic membrane and abundant development of longitudinal muscle columns. The pad of the intracranial artery is a medial structure, located between reduplicated elastic laminae, without an overlying intimal connective tissue. (Resorcin fuchsin-alcian blue. Magnification × 440.)

pressure. The effect of decreased pressure at the origin of collaterals may be augmented by the changes in pressure in the distal vascular beds of nonischemic vs. ischemic myocardium, and by the action of vasodilator drugs. There are no special studies on the effect of vasodilators on the capacity of branch pads or cushions to regulate blood flow in human coronary arteries, but many experimental results suggest that all vasodilators increase flow velocity which may lead to decreased pressure at the origin of collateral vessels. Clinical studies also suggest that coronary steal may occur in any individual with stenotic atherosclerotic plaques submitted to the effect of vasodilater drugs. Clinical studies also revealed that during arteriolar vasodilation produced by drugs, an increase in myocardial infarct size was sometimes recorded due to stealing of the collateral flow away from the ischemic regions.

A coronary steal, subendocardial to subepicardial, is of paramount importance in the natural history of coronary atherosclerosis. It may occur during vasodilation of the vascular bed distal to severe stenosis and may transform a subendocardial ischemia in a transmural infarction, particularly if subendocardial perfusion is reduced for a sufficient period of time. These differences in pressure gradients between subendocardial and subepicardial areas may be associated with those occurring between ischemic and nonischemic regions in other myocardial zones. A steal of coronary flow away from the ischemic area might appear, for instance, in all regions with important cell swelling followed by a passive increase in local vascular resistance.

An additional particular feature of the human coronary circulation seems to be *vascular waterfall*. This term refers to the pressure flow characteristics of collapsible vessels submitted to external compressive forces.[123] It could result from the systolic compression of intramyocardial vessels and could influence the transmural distribution of coronary flow. It might also result from diastolic tissue pressure, acting in concert with arterial smooth muscle tone and venous pressure.[124] According to certain results, diastolic left ventricular pressure appears to be the main determinant of waterfall phenomenon; when left ventricular diastolic pressure increases without a comparable increase in right atrial pressure (a condition which appears in patients with coronary heart disease), a waterfall phenomenon may substantially reduce the driving pressure for myocardial perfusion leading to ischemia and necrosis. If inflow pressure is progressively lowered, intramyocardial flow will stop when inflow and tissue extravascular pressure are the same. A vascular waterfall phenomenon present in epicardial coronary veins was also demonstrated to be able to influence both arterial and venous coronary blood flow.[125] According to certain investigators vascular waterfall formation could represent the mechanism whereby cardiac contraction inhibits regional coronary flow,[126] and a waterfall capacitance model for the coronary arteries has been proposed.[127]

2. Coronary Flow

Some particular data in this field have already been mentioned in the introductory remarks.

The main function of the coronary arteries is to conduct blood to the contracting heart, a constantly exercised muscle that may speed up or slow down from one momemt to another and acts continuously as an efficient pump, using for itself only 5% of the total cardiac output.[128] About 80% of the oxygen requirement of normal heart is used for contraction and about 20% is necessary for viability. The heart has the highest oxygen demand and poorest oxygen supply of any organ in the body, being permanently close to ischemia which rapidly appears in the presence of stenotic atherosclerotic plaques able to reduce this physiologically low flow still further. The development of stenotic atherosclerotic plaques associated with inadequate collateral supply may create stituations in which myocardial cells have sufficient oxygen to remain viable, but insufficient for contraction.

The reduced coronary flow is partly compensated by: (1) a very rich intramyocardial capillary network, more than 4000 capillaries per square millimeter being recorded in the subendocardial regions and (2) the capacity of myocardial cells to extract approximately

75% of the delivered oxygen, in contrast to only 25% removed by the cells of other organs and tissues. Since oxygen extraction in the myocardium is nearly complete and can only be marginally raised by further oxygen extraction, changes in the myocardial energy demands of the human heart can be adequately compensated only by changes in the coronary blood flow.

Coronary blood flow may be expressed by the equation $Q = p/R$, where Q is coronary blood flow, p the gradient between mean aortic pressure (80 mmHg) and mean right atrial pressure (2 mmHg), and R the resistance opposed to coronary flow; this is analyzed in the next section.

Average coronary circulation time is about 4 sec and the following major determinants are involved:

1. Blood pressure at aortic ostia
2. Blood pressure in successive segments of the coronary arterial tree
3. Arteriolar tone (autoregulation)
4. Intramyocardial pressure or extravascular resistance
5. Presence of collateral vessels
6. Blood viscosity

Of particular importance is the *arteriolar tone*, since by means of autoregulation it adjusts coronary flow to myocardial demands, regardless of aortic pressure. This would mean that *any increase in myocardial oxygen demand leads first to coronary arteriolar dilation*, and thereby to augmented coronary blood flow. *The capacity of the coronary arterial tree to augment flow by decreasing arteriolar tone is designated as "coronary reserve"*. Under conditions of stress, *resting flow may increase more than fourfold by this mechanism*. Likewise, owing to the existence of this mechanism, the blood flow does not increase proportionally to the increase in perfusion pressure. Myocardial perfusion depends upon the aortic pressure but, in point of fact, is mainly a function of the resistance of small coronary vessels.[129] Many disturbances in the coronary circulation have as their starting point alterations in coronary reserve; these alterations are reflected by the impossibility of the heart to increase the blood flow in intramyocardial vessels by more than 400% under comparable coronary perfusion presure.

The functional capacity of each vascular segment of the coronary arterial tree is maintained by: the muscular tone and elastic properties of the artery wall, the forces of the distending arterial blood pressure, and the compressing extramural pressure. The latter phenomenon is particular to the heart which, unlike any other organ of the body, seriously impedes its own perfusion while performing its normal pumping function. The heart tries to compensate the low coronary flow, which is only 5% of cardiac output, by a very high oxygen extraction per gram of heart muscle; however, this high oxygen extraction does not occur continuously, because the coronary arterial bed has a fluctuating resistance, dominated by the left ventricular contraction which impedes flow through the ventricular wall. This can be clearly demonstrated in experiments in which left and right coronary artery flow is measured at constant perfusion pressure before and after temporary asystole; with the onset of asystole there is always a dramatic increase in coronary blood flow. In contrast to other arterial beds which show a more or less constant resistance to flow during a cardiac cycle, in the coronary arterial tree a "throttling" effect exists which determines that under normal resting conditions 80 to 90% of the coronary blood flow occurs during diastole, the left ventricular coronary flow being almost entirely a diastolic phenomenon. Since 80 to 90% of the blood flow reaches the contractile tissue during diastole, its duration is an important determinant of left ventricular perfusion.[130,131] The small branches of the left anterior descending and left circumflex arteries are mainly exposed to the intramural pressure generated during left ven-

tricular systole, when there is a sudden decrease in left coronary flow and reversal of flow in some instances. Consequently, the proximal and intermediate segments of the left anterior descending and left circumflex arteries are mainly exposed during systole, not only to pressure from the blood within the aorta, but also to retrograde flow from small intramyocardial branches which are being compressed by the contracting left ventricle. During a part of each cardiac cycle, the hemodynamic stresses in these two major coronary arteries seem to exert an important atherogenic effect which is unique among the other segments of the coronary arterial tree and among the arterial beds of other organs and tissues. Special interest must be paid to the fact that the coronary arterial inflow is minimal during systole and maximal during early diastole, this inflow falling abruptly with the onset of the isovolumetric contraction. On the other hand, the intramural pressure changes in the right ventricle are much smaller than in the left, (the right coronary flow is less affected than the left coronary flow). This systolic cessation of flow in the left but not in the right ventricle is of some clinical interest, for the great majority of myocardial infarcts develop in the left ventricle. In addition, this makes the left ventricle susceptible to all factors that reduce coronary flow. This extravascular systolic pressure is greatest in the inner layer of the left ventricle, and the subendocardial zone occurs as "normally" ischemic.

If heart cells die as a result of prolonged ischemia, they cannot be replaced, since heart muscle cells are not able to multiply after the postnatal period. Consequently, the remaining cells must take over the responsibility of the lost ones, whereas small areas of fibrosis will develop in places previously occupied by contractile elements. For this reason, particular attention must be paid to causes of cardiac cell death and to the ways to protect these cells from ischemic injuries and to enhance their capacity of survival in the face of injury. In this respect, it is important to remember the negative role played by tachycardia. We have already mentioned that 80 to 90% of the blood reaches the contractile tissue during diastole and that its duration is an important determinant of left ventricular perfusion. As the duration of the diastole becomes progressively shorter, the time period in which the intramyocardial vessels of the left ventricle are compressed becomes progressively longer. All reductions in the diastolic intervals may be followed by the onset of cardiac cell injury, closely related to the severity of tachycardia. During psychosocial stresses, exercises, and many other conditions which lead to catecholamine release, the energy requirement of the myocardium increases significantly. The source of this energy derives from the oxidation of high energy phosphates from glycolysis. When tachycardia and the associated conditions produce a diminution of blood flow, not enough oxygenated blood can be carried to the contracting cells to cover their demand and ischemia develops, starting with the subendocardial region of the left ventricle. When discussing the so-called susceptibility of the left ventricle to ischemia and infarction, it seems necessary to re-emphasize the existence of this subendocardial region with its "physiological" deficit in oxygen. This deficit may be accelerated in tachycardic subjects and aggravated by the onset of stenotic atherosclerotic plaques, coronary vasospasm, intravascular emboli and platelet aggregates, coronary steal, etc. The most important in this respect are the stenotic atherosclerotic plaques associated with an inadequate development of collateral vessels in a subject with tachycardia. In such conditions progressive oxygen deprivation is accompanied by the inadequate removal of metabolites. In addition to tachycardia, an important negative role is played by various arrhythmias which produce complex hemodynamic alterations. The cardiac consequences are reflected by changes in the heart rate, the filling and emptying characteristics of the various heart chambers, and the relative timing of atrial and ventricular systole. Nearly any departure from regular sinus rhythm may adversely affect the performance of the heart.[132]

The resulting cyclic reduction in the rate of coronary arterial flow associated with a considerable increase in lateral pressure and wall tension was considered for a long period to be able to produce in the coronary arteries changes similar to those occurring in an artery supplying an atrophic organ.[133]

Myocardial ischemia decreases the systolic throttling effect and tends to allow greater systolic blood flow. On the other hand, an altered myocardium may adversely affect diastolic coronary flow, particularly in the subendocardial region. This may result from an increase in left ventricular diastolic pressure through incomplete relaxation, thus raising intramyocardial pressure during diastole.

The particular hemodynamic and mechanical conditions existing in the coronary arteries could explain the rapid degradation of their internal elastic membrane, starting from the end of fetal life, and the rapid development of a very thick intima during infancy and childhood; these conditions could also help to explain the rapid onset during childhood of the first fibromuscular plaques. They could be also related to the rapid progression of coronary atherosclerotic early lesions toward fibronecrotic or fibrohyaline plaques of possible clinical significance. Therefore, the natural history of coronary atherosclerosis cannot be compared or superposed upon that of atherosclerotic lesions developing in other arterial beds or in experimental models. In addition, it is not possible to overlook that some segments of human coronary arteries are rhythmically subject to torsion, spiral twisting, and bending with every myocardial contraction and are rhythmically compressed by the contracting heart muscle. The mechanical and hemodynamic stresses which act on an atherosclerotic plaque are by far more intense and more complex in the coronary arteries than in other organ arteries or in experimental models.[134] This refers to extramural segments of the major coronary arteries and of the main branch vessels where stenotic or occlusive atherosclerotic plaques usually develop. On the other hand, the contracting heart seems to "protect" intramyocardial vessels from hemodynamic and mechanical stresses, since these vessels appear resistant to atherosclerotic involvement. Even in systemic hypertension, these intramyocardial branches of the coronary arteries are not affected as are the small systemic arteries and arterioles. This may be due to the fact that the intramyocardial vessels are not exposed to the high systolic pressure because they are mainly perfused in diastole.[135]

3. Coronary Autoregulation

Under physiologic conditions, an increase in coronary flow is associated with a decrease in small vessel resistance, resulting in net augmentation of the myocardial supply. The mechanism by which coronary flow is adequately autoregulated at the level of small coronary vessels is not clearly understood and the possible role of branch pads or cushions is often totally neglected.

The coronary arterial tree has the capacity to keep the flow constant by means of small vessel resistance and dilation; with increase of the vascular tone, as perfusion pressure is augmented, the blood flow does not increase proportionally. This "autoregulation" produces changes in the diameter of the resistance vessels, controlled by a feedback mechanism, according to the metabolic demands of the myocardium. Autoregulation is mainly influenced by *local tissue hypoxia*, which appears as an important stimulus to increase coronary flow, associated with the release of *adenosine* from breakdown of the adenine nucleotides.

The potent and widespread vascular action of purine nucleosides and nucleotides was first recognized 60 years ago.[136] Subsequent studies revealed that adenosine mediates the vasodilation seen in various vascular beds under conditions in which oxygen delivery is decreased. Adenosine, a poweful coronary vasodilator, is presumed to connect blood flow with the myocardial metabolic state.[116,137-139] The amounts of adenosine seem to parallel the duration of ischemia, but there are also results which suggest that adenosine is not necessary for regulating resting coronary blood flow.[140]

Both medial smooth muscle cells and endothelial cells have an active adenosine and adenine nucleotide metabolism.[141] Physiologically, the coronary endothelium seems to function as metabolic barrier for interstitially or intravascularly accumulated adenosine. Moreover, the vasodilatory action of adenosine appears to be mediated by the endothelium, smooth

muscle cell relaxation being correlated with the presence of an endothelial cell adenosine receptor.

Of note are also the observations showing that P_1 and P_2 purinoreceptor agonists affect the tone of coronary vessels; removal of the endothelial cells abolishes this relaxation, suggesting that purinoceptors are also located on the endothelium. In addition to adenosine triphosphate (ATP) and its related purines (ADP, AMP, and adenosine), several other vasoactive substances, including acetylcholine, substance P, bradykinin, histamine, and thrombin, can induce vasodilation via receptors located on the vessel endothelium.[142] ATP can cause vasodilation, acting via P_2 purinoceptors located on vascular endothelium, and this action produces the release of an endothelium-derived relaxing factor which diffuses to medial smooth muscle cells and induces vasodilation.[143] Evidence has also been presented for ATP acting as an excitatory cotransmitter with noradrenaline from sympathetic perivascular nerves. Certain results suggest that ATP has a dual function in the regulation of coronary tone: as a vasodilator, acting via inhibitory P_2-purinoceptors located on endothelial cells and as a vasoconstrictor, acting via excitatory P_2-purinoceptors located on vascular smooth muscle cells after release as a cotransmitter with noradrenaline from perivascular sympathetic nerves.[143] Many other factors could play a significant role in coronary autoregulation, in addition to adenosine and related substances. Of special interest seems to be a group of biologically active, lipid soluble fatty acids included in the family of prostaglandins and which possess potent vasoconstrictor and vasodilator properties; in addition, they act as modulators of adrenergic neuroeffector transmission and of the response of smooth muscle cells to various vasoactive agents. The action of prostaglandins is highly dependent on energy-requiring processes, their mechanism of action including changes in the cyclic nucleotide system, in permeability and binding of calcium, in membrane bound sodium-potassium ATPase, and many other changes. Under certain experimental conditions some prostaglandins such as PGF_2 and PGB_2 are vasoconstrictors, others such as PGE_1, PGE_2, PGA_1, PGA_2, and PGB_1 are vasoconstrictors at high concentrations and vasodilators at low concentrations. Among the potential vasoconstrictors of particular importance is thromboxane A_2 (TXA_2).

A basic component of the mechanism of autoregulation is the active tone of medial smooth muscle cells resulting in a gradual state of contraction. Contraction of smooth muscle cells presenting a circular orientation is considered by many investigators to be the main way in which a coronary vessel reacts to stimulation, active vasoconstriction appearing as a non-specific result of a wide variety of stimuli.

The active contractile force may vary from 1.5 to 2.5 $\times$ 10^6 dyn/cm^2 and seems to be mainly stretch dependent. The basal tone is myogenic in that it occurs independently of innervation, associated with stretch and can be considered the most important determinant of coronary resistance.[144]

In spite of the fact that not all pieces of experimental evidence advocate for the regulation of smooth muscle cell contractile activity by means of phosphorylation and dephosphorylation of the myosin light chains, this represents the most widely accepted view. It assumes that in the presence of calcium ions the myosin light chain kinase phosphorylates myosin, allowing the subsequent actin-activation of the Mg^{2+}-ATPase activity of myosin and the occurrence of contraction. When Ca^{2+} is removed the kinase is inactivated and myosin light chain phosphatase removes the phosphate group from the myosin light chain. In an ulterior step the actin-myosin complex dissociates and the vascular wall relaxes. This may be followed by a new cycle in which kinase acts to activate the contractile apparatus and the phosphatase serves to deactivate it.

A suggestion has been made that smooth muscle cells present in the arterial wall as contractile elements are submitted to positive and negative signals which control and regulate contractility.[113] The positive signals are mainly represented by calcium which activates specific protein kinases. These kinases regulate contractility through phosphorylation of

specific target proteins. The negative signals are cyclic adenosine monophosphate (cAMP) and cyclic guanosine triphosphate (cGMP), and the phosphorylation reactions regulated by both cAMP and cGMP are able to inactivate the membrane systems generating positive signals. The formation of cGMP can be stimulated by a factor derived from endothelial cells. This endothelium-derived relaxing factor is released by a variety of neurotransmitters and is a potent vasodilator. In some experimental models cAMP relaxes the smooth muscle cells presumably by affecting the calcium sensitivity of the contractile apparatus, whereas cGMP affects mainly the free calcium concentration. Likewise, in some experimental models membrane depolarization did not appear as a prerequisite for an increase of the cytosolic calcium concentration (neurotransmitters are able to elicit contraction in the absence of a change in membrane potential).

Evidence is accumulating that troponin may be absent in many arterial smooth muscle cells and the contractile proteins actin and myosin differ between striated and smooth muscle; moreover, the major intracellular calcium receptor in smooth muscle cells from vascular walls is calmodulin. An increase in cytosolic calcium activates the contractile apparatus and thereby increases vascular tone as a result of a calmodulin-dependent phosphorylation of myosin and other associated and intricated mechanisms. According to the phosphorylation hypothesis, the calcium-calmodulin complex activates myosin chain 2, and phosphorylation of light chain 2 precedes contraction. This would indicate that phosphorylation of myosin is mainly necessary for the initiation of contraction and represents an important control mechanism for contraction. According to certain results, a rise in free cytosolic calcium and the phosphate content of myosin is necessary for initiation of contraction, but not for maintenance of smooth muscle tone involved in coronary autoregulation. In arterial smooth muscle cells contraction seems to be maintained in the presence of a low free calcium concentration.[113] The maintenance of muscle tone would require additional mechanisms, such as calmodulin-dependent regulation of thin filaments through the protein caldesmon; this would not necessitate phosphorylation or dephosphorylation of myosin light chain 2.[145]

It is important to point out that the electrical depolarization of the smooth muscle cell membrane to open voltage-dependent calcium channels which allows the passive influx of calcium from the extracellular space to the cytosolic compartment was not convincingly demonstrated and remains a subject of debate. Moreover, in certain experiments, differentiation has not been achieved between the electrically stimulated release of a neurotransmitter and the direct depolarization of the plasma membrane.[113]

The complexity of observations must be related to the fact that coronary arteries include "pure" contractile cells, cells which produce regular oscillations of tone and pacemaker cells similar to those of the conducting system of the heart. This last type of cell seems to be mainly localized in longitudinal muscle columns along the border between the media and the thickened intima. Their presence could be related to the occurrence of spontaneous contractions in the samples of human coronary arteries removed on post-mortem examination. These spontaneous cyclical contractions were more clearly revealed in the coronary arteries obtained from recipient hearts of patients receiving cardiac transplantation.[146,147] Post-mortem investigations showed that the spontaneous periodic contractions may be recorded more frequently in the vascular segments in which the degree of atherosclerotic involvement was moderate rather than minimal or severe and in which intimal thickening appeared as circumferential rather than eccentric.[148] A peculiar finding is that in human coronary arteries post-mortem spontaneous periodic contractions did not occur without intimal thickening and, when the thickening reached a certain degree, the incidence of these spontaneous periodic contractions suddenly increased.[148] We also observed that isolated post-mortem human coronary arteries developed periodic contractions within intervals of several minutes in a physiological saline solution, without calcium and without any added vasoactive agent or injury. Human and cattle coronary arteries are conspicuous in showing this spontaneous rhythmic

activity in addition to a basal vasoconstrictor tone. This would indicate the presence of an efficient conducting mechanism between neighboring smooth muscle cells of the coronary media. Of note are also the observations which revealed that these contractions may consist of large amplitude, slow-cycle or low amplitude, fast-cycle rhythms and that in some vascular segments the contractions merged into a state of sustained tone.[149]

In most organs, the smaller vessels within the organ parenchyma are of particular interest because they provide the resistance to blood flow. In the myocardium, these small vessels belonging to the coronary arterial tree may be dilated in response to myocardial metabolic activity and therefore deserve careful attention. The pathophysiologic consequences of this metabolic dilation are sometimes difficult to interpret, particularly in middle-aged man, since the major coronary arteries may restrict flow briefly or persistently due to vasospasm or stenotic atherosclerotic plaques. Whereas a severe luminal insufficiency of a major coronary artery (usually more than 75% vessel narrowing, produced by a stenotic atherosclerotic plaque) is considered to be of possible hemodynamic significance in the absence of adequate collateral vessels, even slight changes in the diameter of small vessels can result in profound alterations in myocardial perfusion.

For this reason many investigators assume that no animal species has a coronary arterial tree that can serve adequately as a model for the physiology and pathophysiology of the human coronary circulation.[149]

An "abnormal" vasodilator reserve was suggested to exist only in man and its presence was related to myocardial clinical manifestations occurring as angina-like chest pain associated with angiographically "normal" coronary arteries.[150] An impairment in coronary reserve specific to man is probably an important contributing factor in the occurrence of both silent myocardial ischemia and anginal pain. This impairment may be produced by the obstruction of large coronary arteries and this was clearly demonstrated in many experimental models; it may be also induced by a failure of coronary reserve following the occurrence of multiple emboli in small intramyocardial vessels, but this is difficult to achieve in experimental models.

The main factors and mechanisms which could influence coronary blood flow are diagrammatically presented in Figure 8.[151]

B. Coronary Innervation

1. General Observations

The coronary arteries and the heart are continuously regulated by the nervous system, a variety of impulses and reflexes being detected which may influence coronary size, coronary resistance, coronary flow, cardiac rate, myocardial contractile force, etc. Of particular importance are high and low pressure baroreflexes, as well as certain chemoreflexes, since they may become abnormal with the development of atherosclerotic plaques. There is good evidence to suggest that certain of these altered excitatory reflexes can contribute to the development of severe arrhythmias, particularly ventricular fibrillation, followed by sudden cardiac death. Similarly, of particular importance are the alterations in baroreceptor discharge activity. This may be related to the fact that afferent fibers in the cardiac sympathetic nerves appear to be solely responsible for signaling the pain of myocardial ischemia; these nerves not only signal pain, but also initiate excitatory cardiovascular reflexes manifested by a complex increase in coronary arterial tone, arterial pressure, small vessel resistance, heart rate, ventricular contractility, myocardial excitability, etc. Consequently, inadequate coronary flow, anginal pain, hypertension, and tachycardia are frequently recorded together in patients with myocardial clinical manifestations induced by coronary atherosclerosis. Many of these patients show profound alterations in the activity of the sympathetic nervous system. It should also be stressed that most deaths due to acute myocardial infarction are associated with an increased activity of the nervous system and augmented levels of blood and urinary catecholamines.

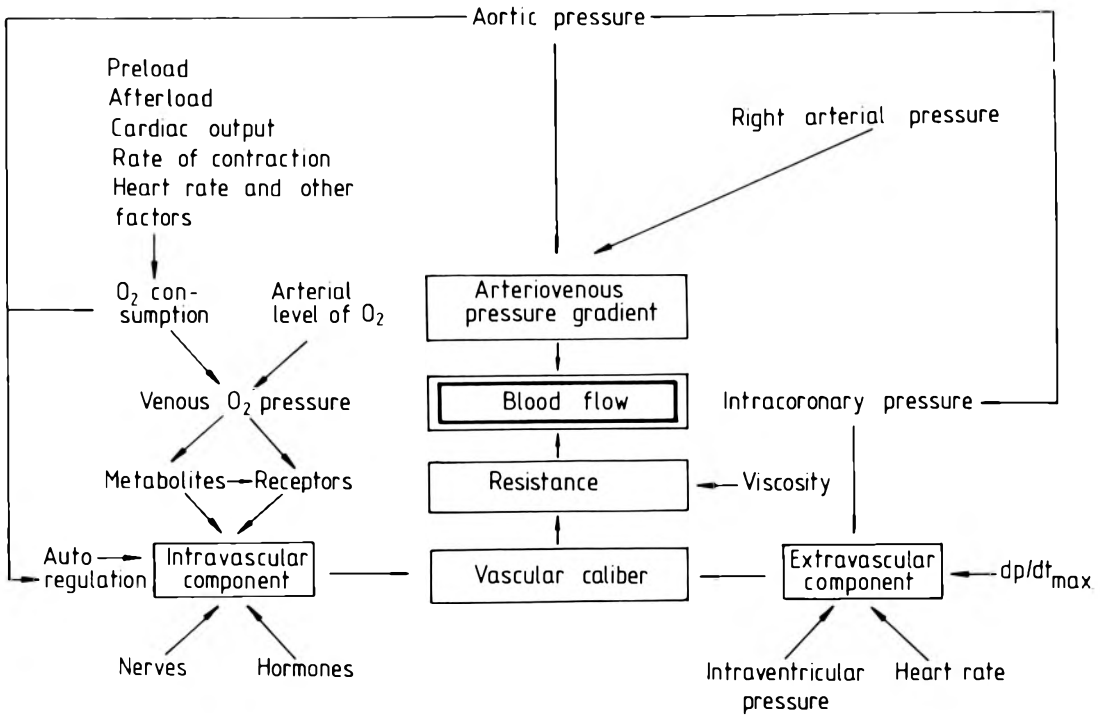


FIGURE 8. Diagrammatic representation of factors influencing coronary blood flow. (From Lochner, W. et al., *Brain and Heart Infarct*, Zülch, K. J. et al., Eds., Springer-Verlag, Berlin, 1979. With permission.)

More than 25 years ago, a view was advanced that the nervous system overactivity which follows stressful conditions (anxiety, exertion, cold exposure, etc.) elicits myocardial ischemia and may lead to cell necrosis.[152] These changes were related to catecholamine release and this release was presented as a key factor responsible for the occurrence of the end stages of the natural history of coronary atherosclerosis. Studies in patients with acute myocardial infarction have shown that urinary catecholemines may be elevated several times. On the other hand certain experimental results suggested that susceptibility to atherosclerosis may be associated with reduced indexes of sympathetic activity.[153] Other experimental results revealed that (1) vagal efferent activity may protect against cardiac electrical instability induced by sympathetic nerves; (2) β-adrenergic receptor blockade may reduce the incidence of sudden cardiac death; (3) the nervous system exerts its complex influences by the modulation of the metabolic pattern of the coronary arteries; and (4) these trophic influences could be more important than vasomotor ones in atherogenesis.

Studies using impregnation and staining techniques have described a rich innervation of the coronary arterial bed.[154-156] In addition, histochemical methods have shown a prevalent adrenergic plexus located between the adventitia and the media.[157] Both sympathetic and parasympathetic nerves may be seen in close association with coronary vessels. In certain studies the sympathetic nerves appeared more diffusely ramified than the parasympathetic ones, the latter being mainly associated with small arteries and arterioles. Structures resembling cholinergic nerve fibers have been located at the periphery of some extramyocardial vessels organized in an adventitial plexus.[158] These cholinergic nerves have been considered to include sensory nerve endings and their presence might be related to the existence of mechanoreceptors in the wall of major coronary arteries.

Chemoreceptors can be identified as perivascular glomoid bodies. The most demonstrative structure in this respect is that located in the immediate vicinity of the left main coronary artery, from which it receives its blood supply.[159] Its general histologic feature resembles that of the carotid body, some of the cells being involved in the production and storage of

amines, others serving as sensory or chemoreceptor sites. The real significance of these particular structures in relation to the natural history of coronary atherosclerosis is difficult to delineate, since very few and inconsistent data exist on the presence in human coronary arteries of cholinergic, purinergic, and possibly peptidergic nerves, in addition to adrenergic nerves which are the dominant autonomic nerve component.

Removal of the left stellate ganglion eliminated the adrenergic fibers that travel downward along the left anterior descending and left circumflex arteries and reduced their vasoconstrictor tone, the net result being an improved myocardial blood flow at the level of the left ventricle.[160] The left anterior descending artery seems to be under the predominant, but not exclusive, control of the left stellate ganglion, whereas the right stellate ganglion contributes to a small portion of fibers. This very important vessel, also called "artery of sudden death", contracts following stimulation of both left and right stellate ganglia.[161] A constrictor tone of the left circumflex artery was also recorded as a result of sympathetic nervous system stimulation and this constriction persisted even in the presence of a significant increase in intravascular pressure.[162] There are also experimental models in which right stellate stimulation was followed by a significant decrease in coronary blood flow in the anterior wall of the left ventricle, whereas left stellate stimulation produced significant diminution in coronary blood flow in the walls of both left and right ventricles.

The control exerted by the sympathetic nervous system was also demonstrated on collateral vessels.[163-165] This would indicate that an abnormal sympathetic vasoconstrictor tone may lead to the extension of preexistent ischemic areas and that the nervous system may be involved in an adequate blood supply at the border of an ischemic area. This might also explain why in some experimental models infarct size was significantly reduced by left stellectomy; in addition, bilateral stellectomy decreased the myocardial susceptibility to ventricular fibrillation. It is tempting to presume that during myocardial ischemia and with the activation of cardiocardiac sympathetic reflexes, a reduction in coronary blood flow to the marginally ischemic areas may occur and can represent a major factor in the occurrence of lethal arrhythmias. In certain experimental models, left stellectomy was found to be the method able to induce a major increase in the ventricular fibrillation threshold, while right stellectomy had the opposite effect, dependent upon an intact left stellate ganglion.[166]

Stellectomy in man produced only partial denervation, and to achieve results similar to those obtained in experimental animals, it seems necessary to perform a high thoracic sympathectomy, removing the first four to five thoracic ganglia. Nevertheless, the arrhythmogenic potential of the left-side cardiac sympathetic nerves cannot be overlooked and many experiments have been reported of ventricular arrhythmias induced by stimulation of the left stellate ganglion. From the point of view of the natural history of coronary atherosclerosis, it is important to emphasize that stimulation of either the right or the left stellate ganglion may facilitate induction of ventricular fibrillation in a subject with chronic myocardial ischemia produced by stenotic atherosclerotic plaques and inadequate development of collateral vessels.

In unanesthetized conscious pigs with occlusion of the left anterior descending artery, ventricular fibrillation was prevented or delayed by reduced stress as a result of adaptation, or by blockade of the frontocortical brain stem pathway traveling through the posterior hypothalamus.[167,168]

There are many examples in the available literature which demonstrate that certain changes in coronary artery innervation may be deeply involved in the natural history of coronary atherosclerosis, particularly in its end stages occurring as angina pectoris, myocardial infarction, or sudden cardiac death. These data reinforce the view that "among the influences that can most quickly and powerfully alter the electrical stability of the heart, its neural control must be placed high on the list".[169]

The pattern of adrenergic innervation with nerves confined to the adventitial side of the

media appears to be suitable for control by both adrenergic nerves and plasma catecholamines. The noninnervated media and thickened intima of the coronary arteries behave as a supersensitive denervated tissue rich in smooth muscle cells.[170] This mainly refers to the major coronary arteries which are sparsely innervated; as the diameter of the vessel gets smaller, the density of innervation increases. Consequently, the peak innervation can be detected in small arteries and arterioles and also in intimal pads or cushions which are densely innervated. Even in such densely innervated vascular segments, the asymmetric arrangement of the nerves leaves most of the smooth muscle devoid of adrenergic fibers and therefore of sites of norepinephrine inactivation. In the noninneurated smooth muscle cells norepinephrine released from sympathetic nerves cannot be inactivated by re-uptake into the nerve terminal following its action on the effector cell membrane. On the other hand, there is a presynaptic inhibition of norepinephrine release from sympathetic nerves by acetylcholine, ATP, and also by adenosine. This represents an important peripheral mechanism of vasomotor control, similar to the negative feedback mechanism by norepinephrine itself.

The concept that each nerve cell can synthesize, store, and release only one neurotransmitter seems to be no longer tenable; the existence of nerves that can synthesize, store, and release more than one pharmacologically active substance is more and more widely accepted. The functional significance of co-storage of transmitters seems to depend on the functional activity of the nerve terminal, the relative rates of release of cotransmitters being influenced by the number, frequency, and pattern of impulses reaching the nerve terminal.

Norepinephrine released from sympathetic nerve terminals continuously adjusts the coronary vascular tone. Although the amount of transmitter released depends primarily on the degree of activation of postganglionic sympathetic fibers, local metabolites and circulating vasoactive substances affect the smooth muscle cells of the media; concomitantly, they may alter the amount of norepinephrine released. Something we find worthy of remark is the role of the presynaptic modulating receptors in the regulation of norepinephrine release. These receptors are located on sympathetic fibers and include presynaptic α- and β-adrenoreceptors, presynaptic serotoninic receptors, and other types of receptors. The sympathetic nerves are endowed with an adenylate cyclase cAMP playing an important role in the regulation of norepinephrine release. Muscarinic agonists, such as acetylcholine, may inhibit this release from neurons by its action on presynaptic receptors, whereas nicotine may have an opposite effect.

In agreement with the view that many receptors have been identified on the prejunctional nerves, it is tempting to presume that various plasma components and local conditions continuously increase or decrease transmitter release. The discovery of new receptors is a frequent finding in the available literature and it appears that almost every endogenous product is related to at least one receptor. Likewise, part of the action of some drugs seems to be due to their prejunctional effects on nerve receptors. The existence of a common mechanism was suggested related to ATP changes, since ATP may be formed, stored, released, and inactivated at an adrenergic nerve terminal. This ATP released from adrenergic nerves together with the transmitter might act as an inhibitory feedback system in vascular epinephrine transmission.

2. Neural Control of Coronary Circulation

The more largely known receptors of the heart and coronary arteries are the α_1 adrenoreceptors; they appear to be coupled to processes that lead to changes in phosphatidylinositol turnover to the release of two or more second messengers which appear important in eliciting coronary vasoconstriction. Most evidence for α_1-mediated vasoconstriction is derived from experiments in which coronary driving pressure was augmented and coronary blood flow remained constant, thereby producing an increase in calculated coronary vascular resistance.[171] Epinephrine and norepinephrine exert their action by altering the concentration of

Ca^{2+} and/or cAMP in the cytosol of arterial smooth muscle cells, and this action is mediated by α_1-adrenoreceptors. As noted before, the occupancy of the α_1-adrenergic receptor enhances the breakdown of phosphatidylinositol diphosphate to diacylglycerol and inositol phosphate by activation of phospholipase C. Evidence is rapidly accumulating that at least three types of channels are involved in cytosolic calcium level and implicity in smooth muscle cell tone and contraction: (1) a voltage-operated calcium channel, (2) a receptor GTP-regulated calcium channel, and (3) a receptor stimulated release of inositol triphosphate. One or two of these mechanisms may prevail in certain coronary segments and may create particular susceptibilities to vasoconstrictor influences. A certain role is also played by α_1-adrenergic stimulation of calcium release from internal stores such as sarcoplasmic reticulum and plasma membranes. In some experimental models this internal release of calcium seems to be responsible for the initial phasic component of contraction which was seen to be succeeded by a slower tonic component dependent on extracellular calcium and involving augmented calcium permeability of the plasma membrane.

Increase in coronary flow required to match the enhanced oxygen demands during sympathetic stimulation can be limited by α_1-adrenergic-mediated vasoconstriction. Experimental results demonstrated that α_1-adrenergic receptor activation reduced the coronary cross-sectional area even in the presence of a marked increase in distending pressure.[172] Fortunately, in man, activation of cardiac sympathetic nerves under physiologic conditions increases myocardial performance. It induces coronary metabolic vasodilation which competes with the direct α_1-adrenergic vasoconstrictive effect of sympathetic activation. During exercise this vasodilation prevails, the result being an adequate blood supply to the contracting heart. Only in a limited number of cases may the competition between local metabolic vasodilation and α_1-adrenoreceptor vasoconstriction result in diminished myocardial supply, and this could be associated with anginal pain. In such cases, it is tempting to presume that the α-receptor constrictor influence not only limits the increase in oxygen delivery, but also augments capillary oxygen extraction as a result of a very high myocardial oxygen consumption.[173]

The competition between α_1-adrenergic constrictor influence and metabolic vasodilator influence is but an oversimplified view. This competition is far more complex, involving the intervention of many other receptors and vasoactive substances.

The role played by α_2-adrenoreceptors, for instance, in the maintenance of coronary tone seems also to be important. α_2-Receptors serve mainly to mediate the effect of catecholamines; prejunctional α_2-adrenoreceptors exert a negative feedback on the liberation of norepinephrine.[174] These prejunctional receptors belonging to the α_2-subtype are sensitive to the neurotransmitter itself and inhibit norepinephrine release during nerve stimulation. The receptors of α_2-subtype are coupled to a specific guanosine triphosphate-binding protein which inhibits the activity of adenylate cyclase. Some of the receptor-operated calcium channels seem to be regulated by such proteins. α_2-Adrenoreceptors are of particular importance not only when present on nerve endings, but also on the surface of smooth muscle cells of small coronary vessels. On nerve endings they are prejunctional and inhibit neuronal release of catecholamines. On small coronary vessels their stimulation produces complex effects which contribute to maintenance of the muscular tone. Therefore, the presence of α_2-adrenoreceptors is mainly important at the level of small arteries and arterioles and at the level of the precapillary sphincters. These effects compete with those of prejunctional α_1-receptors which produce norepinephrine release and contraction of smooth muscle cells.[175] Many investigators correlated the subdivision of α-adrenergic receptors in 1 and 2 subtypes according to these specific actions and also according to their pre- or postjunctional topography.

An important role in coronary vasomotricity is played by β_1- and β_2-adrenoreceptors. They activate adenylate cyclase when stimulated, leading to the generation of cAMP, the activation of cAMP-dependent protein kinase, and the phosphorylation of many cellular

substrates. Stimulation of the β-adrenergic receptors by norepinephrine not only activate adenylate cyclase, but also increases cytosolic cAMP levels and decreases vascular smooth muscle tone. This stimulation initiates the exchange of guanosine diphosphate with GTP on a GTP-binding protein which then dissociates into subunits, certain of these subunits stimulating adenylate cyclase. In the next step, cAMP decreases smooth muscle tone either by augmenting the uptake of cytosolic calcium into stores, or by decreasing the calcium sensitivity of the contractile apparatus. Evidence is accumulating that stimulation of β-adrenergic receptors increase cAMP levels and the activity ratio of cAMP-dependent protein kinase in the coronary arteries.[176] These might also influence platelet aggregability in the coronary arterial tree, especially aggregated platelets at the site of stenotic atherosclerotic plaques, leading to cyclical changes in coronary blood flow. These phenomena were abolished by stellectomy,[177] being related to a catecholamine-mediated fluctuation in platelet aggregability and in receptor and adenylate cyclase activities.[178]

The density of β-adrenergic receptors appears to be inversely related to the magnitude of stimulation of the respective receptors by agonists. A high sympathetic nervous activity is associated with a low density of β-receptors and vice versa. This decrease in receptor number has been termed down regulation, whereas their increase is called up regulation. In essence, the higher the level of sympathetic nervous activity (and implicity the greater the concentration of catecholamines), the more down regulation occurs — the number of β-receptors tend to diminish more and more. In the ischemic myocardium, the number of β-receptors increase in association with a marked reduction in local norepinephrine concentration. Blockade of β-adrenergic receptors also leads to an increase in receptor density or up regulation. On the other hand, patients with coronary heart disease may occasionally develop unstable angina pectoris or even myocardial infarction after sudden withdrawal of the agents blocking β-adrenergic receptors. This syndrome was mainly related to hyper-responsiveness of β-adrenergic receptors to stimulation by catecholamines, since the number of these receptors usually augments after prolonged periods of treatment with β-adrenergic-blocking drugs. The most broadly discussed is the propranolol withdrawal syndrome, which is considered to be a clinical entity and which occurs in a minority of patients treated with β-receptor-blocking drugs. When severe coronary atherosclerotic involvement exists, the increase in coronary vascular resistance is amplified and the effects of β-adrenergic receptor blockade may appear more profound, with more marked reduction in coronary blood flow secondary to an additional increase in small vessel resistance. Available evidence suggests that α-adrenergic stimulation by α-agonists, as well as sympathetic stimulation after β-blockade, may produce a luminal reduction in the coronary arteries as severe as that of stenotic atherosclerotic plaques.[172,179]

The coronary arterial bed seems to include many β_1-adrenoreceptors, like the myocardium, and few β_2-adrenoreceptors which prevail in other organ arteries.[180] The topographic distribution of receptors in human coronary arterial tree and their prevalence in various vascular segments, however, remains a subject for future research. The significance of this prevalence and quantitative assessment is questioned by the views which assume the existence of a receptor interconversion. According to these views, there is only one adrenergic receptor type in the coronary arterial wall and its action seems to be influenced by the local environment. Under particular conditions the influence of receptors of the α-type prevails and a vasoconstriction is present; under other particular conditions in the same vascular segment, the influence of receptors of the β-type prevails and a vasodilation is present. In addition to adrenergic receptors the possible influence of other types of receptors must be taken into consideration: the dopamine vascular receptor is also involved in vasodilation and able to counteract the constrictor influences,[181] whereas activation of serotonin receptors may enhance contraction.[182] There are also results which suggest that different subtypes of receptors are involved in diffuse vs. focal constriction.[183]

Ergonovine, methoxamine, or clonidine may provoke coronary spasm by acting as pre- and postsynaptic α-receptor agonists; on the other hand, the administration of α-receptor antagonists (phentolamine or phenoxybenzamine) may reverse spasm. Additional data on this topic are given in the next sections.

A number of lines of evidence support the view that endogenous products of arachidonic acid cyclooxygenase metabolism, such as prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) possess marked vasodilator potency. Such prostaglandins act as local hormones. Once released they are able to counteract vasoconstrictor influences exerted by the stimulation of α-adrenergic receptors. The activation of adrenergic nerves increases the formation of PGE_2, which may modulate the sympathetic constrictor influences by inhibiting norepinephrine release. The local production of vasodilatory prostanoids opposes vasoconstriction induced by sympathetic nerve stimulation and reduces myocardial ischemic events. There is now wide agreement that this production must not be inhibited (for instance, by indomethacin) since vasoconstrictor influences on the coronary arteries of the nervous system will increase. Moreover, the diet must include essential fatty acids (EFAs) which act as precursors of prostanoids. There is at present much interest in the results which show that an increased amount of dietary linoleate (sunflower or fish oils) is associated with a diminution of the vasoconstrictor influences. On the other hand, these vasoconstrictor influences are augmented when TXA_2 is released from platelets during aggregation. TXA_2 is synergistic with serotonin in inducing coronary contraction and in potentiating the response to sympathetic stimulation or to norepinephrine. Enhanced TXA_2 activity may either precede or follow myocardial ischemia and in both circumstances it augments vasoconstrictor influences and aggravates the consequences of the ischemic episode.

An outstanding observation is that the response of arteries to norepinephrine, serotonin, and other vasconstrictor agents may depend on a "balance" between direct constrictor actions on medial smooth muscle cells and the dilator signal generated by endothelial cells. Removal of the endothelium could sensitize the smooth muscle cells to vasoconstrictor agents and thus amplify their contractile effects.[184] On the other hand, in the presence of an unaltered endothelium, isolated arterial rings or strips relax to acetylcholine, bradykinin, or ATP by an endothelium-dependent mechanism.[185] According to this view, all endothelial alterations in the coronary arterial bed may promote an enhancement of various constrictor responses. Moreover, the endothelium of small intramyocardial vessels may modulate the constrictor influences of circulating or local neurally released amines. A "defect" in endothelium vasodilator function could also explain a paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. Whereas in segments of normal coronary arteries, acetylcholine caused a dose-dependent dilation; in segments of atherosclerotic coronary arteries it produced vasoconstriction.[186] An additional important observation was that coronary angiography may detect cases with "normal" or "almost normal" coronary arteries showing different types of responses to acetylcholine along the vessel course.[187] A plausible explanation seems to be the heterogeneity in receptor population along the course of vessels of the coronary arterial tree.

In essence, the prevalent opinion assumes that while small coronary vessels are mainly regulated by changes in myocardial metabolic demands, the whole coronary arterial tree is responsive to neural stimuli. The most striking changes can be observed with the augmentation or withdrawal of α-adrenergic receptor constrictor tone of the major coronary arteries. This sympathetic α-adrenergic receptor-mediated coronary vasoconstriction can be detected even in the presence of advanced atherosclerotic plaques. Moreover, a progressive unmasking of sympathetic vasoconstriction may be recorded with increasing severity of stenosis. Activation of cardiac sympathetic nerves in the presence of severe atherosclerotic plaques is thus an additional factor which decreases coronary blood flow and aggravates myocardial ischemia.[188] This augmented sympathetic vasoconstrictor tone could also represent a critical

factor in the onset of platelet thrombi usually superimposed upon stenotic atherosclerotic plaques. It is tempting to presume that stimulation of the sympathetic nervous system could be involved in both thrombi formation and disintegration at the site of stenotic atherosclerotic plaques, leading to cyclical changes in coronary blood flow.[177,178]

The progressive unmasking of sympathetic vasoconstriction with the increasing severity of atherosclerotic involvement is usually associated with a decrease in coronary reserve. α_1-Postjunctional adrenergic receptors seem to be particularly involved in this abnormal tone of small intramyocardial vessels. It could lead to a continuous shift from diminution in coronary resistance to increase in coronary resistance to cardiac sympathetic nerve stimulation. When the vasoconstrictive effect of sympathetic stimulation gradually overcomes the metabolic dilatory mechanism, a significant decrease in coronary reserve can be recorded. This may be followed by the onset of areas of myocardial ischemia in the regions supplied by stenotic atherosclerotic arteries, particularly in the absence of a well-developed collateral circulation. Vascular β-adrenoreceptor activity continued to reduce the constriction produced by α-adrenoreceptors; pharmacologic blockade with propranolol is able to abolish this dilator action. When distal to a coronary stenosis the vascular bed exhausts its dilatory reserve, and either asymptomatic or symptomatic myocardial ischemia are likely to occur. In these individuals, activation of cardiac sympathetic nerves acts as a major risk factor for coronary heart disease since it increases myocardial performance and oxygen supply. When this sympathetic activation is very intense and prolonged, patchy subendocardial necrotic areas can be detected. A particular step in the natural history of coronary atherosclerosis seems to be represented by the period in which a passage from a prevalent vasodilation to a prevalent α-mediated vasoconstriction occurs in patients with stenotic atherosclerotic plaques and a reduced coronary reserve distal to stenotic lesions.

Two decades ago, not only was the ability of the sympathetic nervous system to cause coronary vasoconstriction demonstrated, but also that this vasoconstriction can be reflexely elicited through carotid sinus activation.[189] The reflex activation of coronary vasoconstriction, dependent upon the sympathetic nervous system, was shown to be blocked by α-adrenergic antagonists and enhanced by β-adrenergic antagonists. In many studies emphasis has been placed on the reflex coronary vasoconstriction as the result of a cold pressor test, a method used to reveal latent or silent myocardial ischemia produced by coronary atherosclerosis. The occurrence of coronary vasoconstriction can be reflexely elicited and this stimulated many investigators to search for the starting point of these reflexes. Carotid sinus hypotension resulted in reflex sympathetic α-receptor coronary vasoconstriction; this reflex vasoconstriction seemed to be independent of changes in myocardial oxygen metabolism or changes in aortic pressure.[190] The cholinergic coronary vasodilator pathway could be activated by stimulating chemosensitive vagal endings in the lung.[191] Coronary chemoceptors seem to be especially vulnerable to ischemia since they have a very high metabolic rate. Ischemia of the chemoceptors might explain vasoconstrictor influences leading to anginal pain and hemodynamic changes such as those occurring in unstable angina pectoris.[192,193] Likewise, ischemia of chemoceptors might trigger coronary and systemic hypertension.

Figure 9[194] tries to summarize the possible site of actions of autonomic neurotransmitters in the coronary arterial tree. Many "gray areas" continue to persist:

1. The prevalence of local metabolic changes or of the autonomic nerves in governing the coronary artery tone
2. The distribution and types of adrenoreceptors in various segments of the coronary arterial tree
3. The effect of activation of these receptors, not only on smooth muscle cells of the media, but also on the coronary blood flow at rest and during exercise
4. The influence of prejunctional receptors on the amount and type of neurotransmitter released

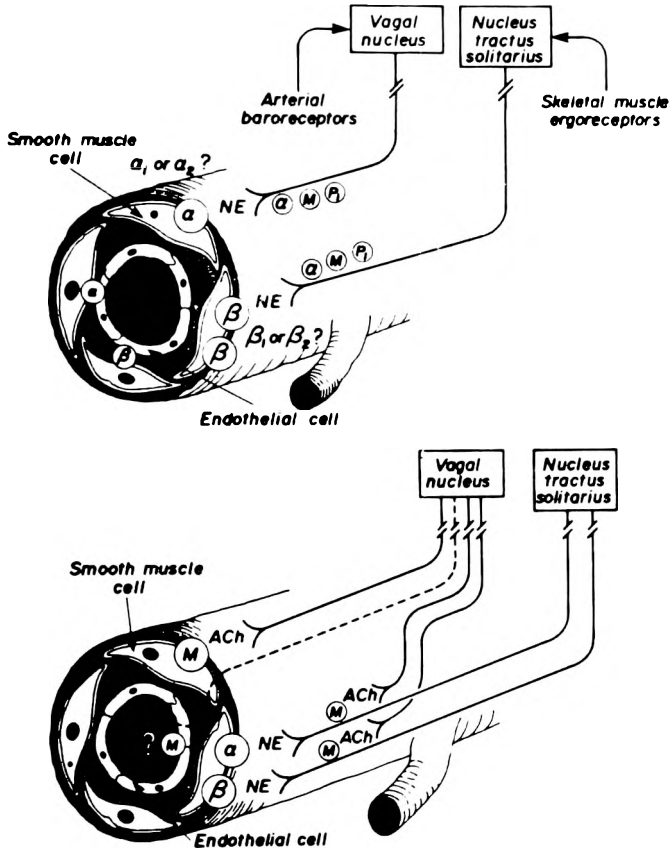


FIGURE 9. Possible site of actions of autonomic neurotransmitters in the coronary arterial wall. (Top) Adrenergic nerves. The amount of norepinephrine released depends not only on the degree of activity of the higher centers, but can also be modulated at the prejunctional level. The norepinephrine released can activate adrenergic receptors on the smooth muscle cell membrane. (Bottom) Cholinergic nerves. The released acetylcholine may act solely at the adrenergic prejunctional level by switching off the release of norepinephrine. Alternatively, it may activate muscarinic receptors on smooth muscle cells to cause either relaxation or contraction. The possibility has not been ruled out that acetylcholine released from cholinergic neurons may trigger endothelium-dependent relaxation. (α) α-Adrenoceptor, (β) β-adrenoceptor, (M) muscarine receptor, (ACH) acetylcholine, (NE) norepinephrine. (From Shepherd J. T. and Vanhoutte, P. M., *Fed. Proc.*, 43, 2855, 1984. With permission.)

5. The direct action of cholinergic fibers on smooth muscle and endothelial cells and the role of these fibers to modulate the exocytic release of norepinephrine
6. The role of the autonomic nerves when centers of the brain and cardiovascular reflexes are activated[194]

It could also be of interest to remember here that coronary arteries undergo spontaneous cyclical contractions, occurring as a genetically transmitted peculiarity.[146-149] These spontaneous cyclical contractions were recorded in post-mortem material and in the coronary arteries obtained from recipient hearts of patients receiving cardiac transplantation.

3. Nervous System and Coronary Vasospasm

The existence of coronary vasospasm has been largely accepted, along with its possible

roles in myocardial clinical manifestations and in silent myocardial ischemia. Coronary vasospasm is an unusually violent contraction of a small or large segment of a major coronary artery which interferes with myocardial blood supply, the most dramatic and usual consequence being anginal pain. This interference with myocardial blood supply documented coronary vasospasm as a pathogenetic mechanism of angina pectoris, myocardial infarction, or sudden cardiac death. This documentation is mainly based on angiographic findings, as coronary vasospasm occurs as a local and transient reduction in the caliber of an apparently normal or slightly affected major coronary artery or by significant worsening or total occlusion of a preexisting stenosis. It may be iatrogenic, spontaneous or induced, single or multiple, and may involve one or more vessels. When the spasm is present in a large vascular segment it may appear similar to a diffuse vasoconstriction. The site of iatrogenic spasm is almost always the first centimeter of the right coronary artery, being triggered by the catheter tip. The sites of spontaneous spasm are, in decreasing frequency, the right coronary artery, the left anterior descending artery, and the left circumflex artery. Spontaneous spasm, like spasm induced by a drug, produces ECG changes and anginal pain, whereas the iatrogenic spasm is not accompanied by ECG changes or anginal pain. The spontaneous or drug-induced spasm tends to completely obstruct the vessel lumen, causing total angiographic disappearance of the downstream bed in the affected artery. This may be associated with rhythm disturbanes, such as ventricular tachycardia or fibrillation, or even atrioventricular block.

An excised coronary artery which has shown spasm on an ergonovine provocation test shortly before excision might present important alterations of vascular nerves.[195] Randomly selected vascular nerves revealed distinct cell debris in epi- and endoneurium and vacuolar degeneration of unmyelinated fibers. The presence of cell debris may provide evidence of cellular abnormalities in vascular nerves and suggests an association of coronary artery nerve injury and increased spasticity of the vessel.[195] This emphasizes the importance which must be placed on cardioneuropathies in the etiopathogenesis of coronary atherosclerosis and coronary heart disease.[104] The existence of only endothelial injury could be a simplified view if the presence of an associated nerve injury is overlooked. This nerve injury not only may produce spasm, but it may be the starting point of abnormal impulses preponderantly relayed along sympathetic nerve fibers through the cardiac nerves and to the paravertebral chain of cervical and thoracic ganglia. The next step seems to be the spinothalamic tract of the spinal cord and finally the posterolateral and ventral nuclei of the thalamus.

An intriguing question is why spasm does not appear in the coronary arteries of children and adolescents and even of young adults and why it represent a problem of mature adults only. The well-developed and apparently unaltered muscular media of young individuals is not prone to produce spasm. This suggests that a prerequisite of spasm is a coronary artery which has acquired an abnormality as it has aged.[196] Spasm appears in "hypersensitive vessels" in which it could be induced by a variable combination of triggering stimuli, particularly nervous system stimulation.[197] There is also good evidence to suggest that coronary atherosclerotic involvement is associated with the alteration of vasomotor activity, exhibiting a focal character, which may lead to a dramatic decrease in luminal size consistent with spasm.

The α_1-adrenergic receptors seem to be mainly involved in diffuse coronary vasoconstriction, whereas the α_2-adrenergic receptors are more clearly associated with spasm occurrence.[183] Ergonovine, methoxamine, and clonidine act as α_2-agonists and produce spasm in the coronary arteries; on the other hand, phentolamine and phenoxybenzamine act as α_2-antagonists and reverse spasm. The activation of serotonin receptors could also be involved in coronary vasospasm, as well as the combined administration of epinephrine and propranolol.[182,199]

In addition to ergometrine or ergonovine, an amine alkaloid of ergot which is largely used to provoke coronary vasospasm, many other substances can produce focal acute vasocon-

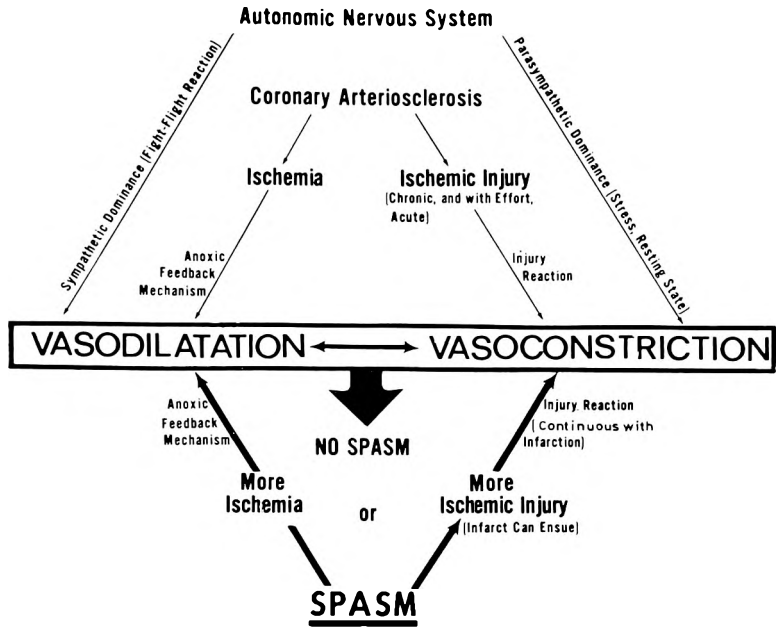


FIGURE 10. Suggested interrelations between nervous system and myocardial ischemia. Spasm causes a new dynamic balance with higher levels of ischemic injury. (From Hellstrom, H. R., *Br. Heart J.*, 41, 426, 1979. With permission.)

striction: epinephrine, norepinephrine, acetylcholine, and TXA_2. Spasm can also be induced following magnesium deficiency. Hypercontractility of medial smooth muscle cells could also be attributed to the accumulation of vasoconstrictor mitogens and leukotrienes and high local concentrations of blood-borne vasoconstrictors in areas of neovascularized atherosclerotic plaques.[198] An additional mechanism involved in this process seems to be a deficiency of the vasodilator prostacyclins and of an endothelium-derived relaxing factor. All evidence points to the fact that an enhanced activity of the autonomous nervous system may cause coronary vasospasm as a result of nerve injury, of an increased level of circulating or local catecholamines, and of the presence of an atherosclerotic lesion with or without clinical significance. A cyclic variation in the sympathetic tone was suggested to exist, spasm occurring only when this imbalance acquired a more general character, possibly at the hypothalamic level. A view was posited that the onset of spasm would reflect an adrenergic imbalance which might produce an asymptomatic stellate ganglion activation, asymmetric activity, excessive unilateral ganglion discharge along cardiac nerves, and selective coronary focal constriction.[200]

A further point of interest is that the coronary arteries contain surprisingly high amounts of serotonin and histamine, the levels of histamine being greatly increased in the coronary arteries of subjects with myocardial clinical manifestations. Histamine has also been shown to cause focal spasm and is used successfully in a provocative test to induce spasm in patients with suspected angina pectoris.[201]

From the viewpoint of the natural history of coronary atherosclerosis, coronary vasospasm is important as a contributing factor in the onset of the end stages of this natural history.[196,197,202-204] This is suggested in Figure 10.[203]

In essence, a new factor of major importance in the natural history of coronary atherosclerosis must be taken into consideration. This factor, partly of genetic origin, might be responsible for the variability of the adrenergic, cholinergic, serotonin-related, and other control mechanisms. The variability is reflected in the innervation pattern, the prevalence of some nerve terminals, the density ratio of α- to β-adrenoreceptors, the density of other types of receptors, and in many other local conditions which are genetically determined in

each individual.[205] The real value of the above-mentioned factor is very much demonstrated in patients with transplanted, and therefore denervated, hearts which respond in different and sometimes inadequate ways to the increased myocardial oxygen demand.

C. Coronary Microarchitecture

1. General Observations

"Variation is the hallmark of the coronary blood vessels."[206] This statement refers not only to coronary anatomy, but also to coronary histology. Structural variations are usually seen at different levels of the same vascular sample and this can be related to the different susceptibilities of various segments of the coronary arterial tree to both age-related changes and atherosclerotic involvement. Each segment of the coronary arterial tree seems to be unique in its relation to various types of atherogenic agents, so that any given region cannot be used as a suitable model for another. The data presented in the previous pages also demonstrate that the physiology and pathophysiology of human coronary arteries seem to be particular and cannot be compared with those of other arterial beds. It is not possible to understand the particular feature of the coronary circulation by looking into the circulation of the brain, kidney, or liver. Likewise, it is not possible to understand the development, remodeling, and structural organization of various segments of the coronary arteries by looking into the histology of other arterial beds. Unfortunately, the textbooks of anatomy, histology, pathology and cardiology overlook the existence of all these particular features of the coronary arterial tree. They have drawn the attention of only a few investigators and this may account for the scarcity of data from the available literature discussed in the following pages.

All studies of the microarchitecture of human coronary arteries have as a starting point the pioneer investigations on arterial smooth muscle cells of the eminent pathologists Daria Haust and Robert Wissler.[207,208] The 1960s provided us with many advances which have helpted to delineate and analyze the element involved in coronary intimal thickening and in the proliferative phase of atherosclerosis. Many advances came with the development of electron microscopy and its application to atherogenesis and atherosclerosis research. The presence of a large number of cytoplasmic myofilaments, numerous pinocytic vesicles along the plasma membrane, and a limiting basement membrane were considered valuable ultrastructural proofs that the respective element is a smooth muscle cell. These intimal smooth muscle cells appear to be multifunctional, able to contract, to exert concomitantly several metabolic activities, and to synthesize various types of enzymes and connective tissue macromolecules. They also migrate, passing from the media into the intima, particularly into the subendothelial region where they can be seen starting from fetal life. They also give rise to branch pads or cushions and to diffuse thickened intima. Light miscroscopic aspects suggesting that medial smooth muscle cells migrate into the intima can be often seen on isolated tissue sections of the coronary arteries of children (Figure 11). This particular mobility also explains the well-known colonization with smooth muscle cells within or on arterial transplants or protheses, followed by the development of a pseudointima in which proliferative lesions may occur while associated with lipid and fibrin deposition. In experimental animals, cholesterol feeding considerably enhances this capacity to migrate from the media into the intima; a similar enhancement was observed after repeated breeding and after the introduction of threads into the lumen and other types of vascular injury.

Observations on about 500 coronary arteries from subjects ranging in age from prematurely newborn to 90 years, led to the concept that the movement of medial smooth muscle cells into the intima is a prerequisite for the onset of atherosclerotic lesions and also for the progression of these lesions.[209] Media seems to play a significant role in atherogenesis by populating the intima with smooth muscle cells. Once medial cells enter the intima, non-atherosclerotic diffuse intimal thickening is initiated. Subsequently, intimal thickening pro-

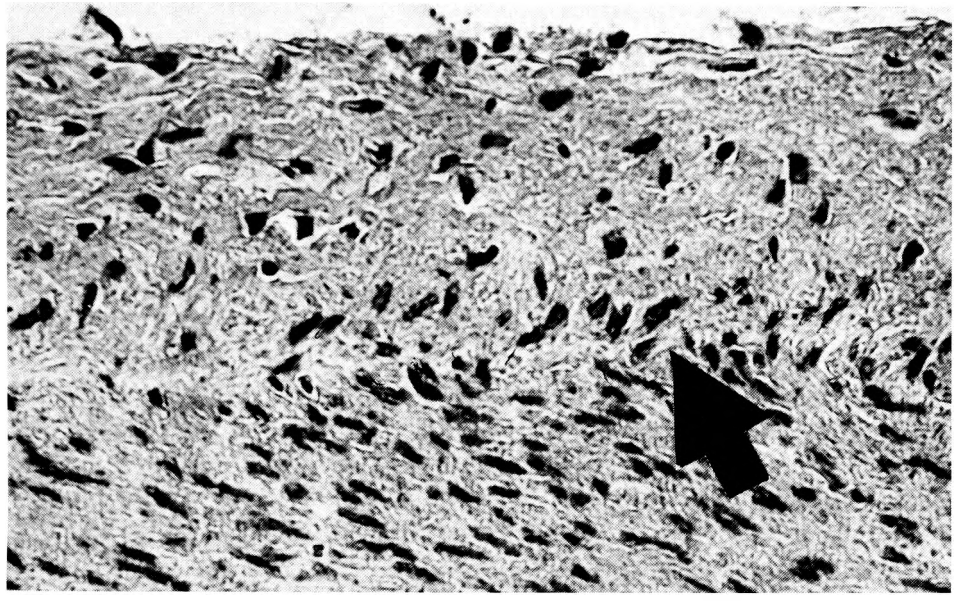


FIGURE 11. Proximal segment of the left anterior descending artery of a 5-year-old male child. Suggested migration of medial smooth muscle cells into the intima through breaks of the internal elastic membrane (arrow). (Toluidine blue 0.1%, pH 5.0. Magnification × 440.)

gresses as the migrated cells proliferate. There are studies which consider migration from the media into the intima not only a prerequisite for intimal thickening, but also a critical point of the formation of atherosclerotic plaques, the most direct evidence being offered by cell kinetic studies.[210,211] Emphasis has been also placed on the heterogeneity of arterial smooth muscle cells.[212,213]

When smooth muscle cells migrate into the intima, they become mainly oriented toward the elaboration of connective tissue macromolecules. This elaboration is activated by all types of injuries acting on the endothelium and the subendothelial zone and necessitating reparative processes. This prevalence of the secretory function is reflected in a progressive loss of myofibrils and a progressive development of the Golgi apparatus and endoplasmic reticulum. In addition, some of the medial cells migrating into the intima act as macrophages and appear as foam cells on light microscopic examination.

The fact that the response to an apparently similar type of injury is reflected by various functional orientations of intimal smooth muscle cells supports the view that they are both contractile elements and multifunctional mesenchymal cells.[208]

There is at present much interest in the observation that the medial smooth muscle cells which have migrated into the intima exhibit a greater phenotypic variability than the smooth muscle cells of the media.[214-217] Likewise, these cells of the intima are able to give a more complex differential response to atherogenic stimuli than the medial cells, according to their phenotypic state. These phenotypic modulations of the smooth muscle cells from the contractile toward the synthetic state appear in certain experimental conditions as a prerequisite for proliferation which gives rise either to thickened intima or to fibromuscular atherosclerotic plaques.

Ultrastructurally, cells in the contractile state have their cytoplasm filled with myofilaments, whereas cells in the synthetic state show scattered, peripherally located bundles of myofilaments, and the bulk of the cytoplasm is filled with organelles involved in the synthesis of ground substance and fibers. Similarly, the activation of PGI_2 production by smooth muscle cells seems to be associated with their phenotypic expression. Reversible and irreversible synthetic states were suggested to exist; only the cells in the irreversible synthetic

state were able to respond immediately to mitogens. Likewise, they are susceptible to lipid accumulation, and they metabolize lipoproteins and accumulate cholesteryl esters. Selection from among a smooth muscle cell population containing such a proliferogenic subpopulation could account for the monoclonal phenotype of some atherosclerotic plaques, or for the monoclonality arising gradually as the coronary atherosclerotic plaques evolve.

In essence, the medial smooth muscle cells which migrated into the intima seem to exist within a large spectrum of modulations. At one end is the smooth muscle cell, the major function of which is vascular contraction and which may be implicated in coronary vasospasm. This cell contains many thick (myosin) and thin (actin) myofilaments, it does not normally divide and does not synthesize appreciable matrix. At the other end is the element engaged in intimal matrix secretion, intimal thickening, and the development of fibromuscular atherosclerotic plaques. This element usually contains thin but no thick myofilaments, large amounts of rough endoplasmic reticulum, and free ribosomes; it synthesizes large amounts of ground substance and fiber components, is not contractile, but is capable of division and responds immediately to mitogens. After multiple cell divisions some of these elements cannot return to the contractile state.[214] These large variations of phenotype modulation were mainly observed in primary cell culture; smooth muscle cells grown in vitro express three distinguishable forms of phenotype: contractile, reversible synthetic, and irreversible synthetic.

A marked heterogeneity was detected among these states with respect to lipoprotein metabolism.[214-216] Arterial smooth muscle cells with the morphologic features of contractile element show a very poor capacity to bind and degrade very low density lipoprotein (VLDL) and low density lipoprotein (LDL) macromolecules. This was associated with the minimal accumulation of intracellular cholesterol and insignificant morphologic evidence of the increase in lipid. On the other hand, the cells in the irreversible synthetic state showed the significant binding capacity of LDL and substantial cholesteryl ester formation and accumulation. This high capacity of smooth muscle cells in the irreversible synthetic state to bind and accumulate lipoproteins in contrast with the low capacity of contractile cells may be relevant to atherogenesis.

Smooth muscle cell cultures of arterial intima and media also offered the possibility to fractionate these elements into two subpopulations, depending upon the capacity of adhesion and on their patterns of growth. The high adhesive cells grew in a monolayer fashion, while the low adhesive cells showed a tendency to multilayered growth. This population capable of multilayered growth seems to be involved in the repair and remodeling of the artery.[218]

Also of note are certain changes in the smooth muscle cell cytoskeleton. When the cells migrate from the media to the intima where they replicate, important quantitative and qualitative changes seem to occur as concerns actin, vimentin, and desmin.[219] The switch from α- to β-actin predominance could represent a reliable marker for replicating smooth muscle cells and, in particular, of smooth muscle cells involved in atherogenesis. In atherosclerotic lesions, a predominance of actin in the β-isoform was detected within the cells, while smooth muscle cells of the normal media largely contain actin in the α-isoform. Likewise, the smooth muscle cells of a neointima formed 2 to 3 weeks after endothelial injury contained a predominance of the β-isoform of actin and only vimentin, whereas the normal media contained the α-isoform of actin and both vimentin and desmin filaments.[220,221]

Smooth muscle cell proliferation in the coronary arterial wall might be influenced or even regulated by means of chalones. These are tissue-specific mitotic inhibitors and act at two points in the cell cycle, inhibiting entry into mitosis and serving as a switch between cell division and maturation. In the tissue culture of arterial smooth muscle cells, chalones prevented the phenotypic modulation from the contractile to the synthetic state.[214] It seems plausible that the arterial wall itself, by production of chalone-like substances, controls the phenotype of smooth muscle cells and implicitly its capacity to respond to mitogens or

mutagens. According to other views, in the normal vessel wall smooth muscle cells would exist in a growth-inhibited rather than in a passive state awaiting the arrival of a mitogen.[98] These mitogens may come from the flowing blood, such as those produced by platelets or by macrophages, or they might be represented by LDL macromolecules from hyperlipemic plasma.

Endothelial cells produce both growth inhibitors and stimulators of smooth muscle cells and some results were mainly centered on a heparin-like macromolecule which seems to exert a significant inhibitory effect on smooth muscle cell growth.[222] As long as smooth muscle cells are surrounded by chalone-like substances in adequate amounts, the respective cells are maintained in the contractile phenotype, unable to proliferate and to metabolize lipid. It is tempting to think that certain injuries to the endothelium may be associated with a reduction in the synthesis of these chalone-like substances and with a modulation from contractile to synthetic phenotype of the cells located in the respective region. Such cells would become susceptible to the action of mitogens. If the endothelial injury is sustained and severe, some smooth muscle cells will remain in the synthetic phenotype for a considerable period of time and the vascular region would appear susceptible to atherogenic agents. Such susceptible regions are usually intimal pads and cushions and areas with a very thick intima.

All the above-mentioned data derive from in vitro studies and some investigators suggest that caution should be used in trying to predict the functional capability of a smooth muscle cell simply from its morphological appearance.[223]

Based on this capacity of smooth muscle cells to proliferate, many similarities have been suggested to exist between atherosclerosis and arterial hypertension. Both are characterized by this capacity of smooth muscle cells to proliferate in the intima of large and medium-sized arteries in atherosclerosis, the media and intima of small arteries, and in arterioles in hypertension. Both diseases may lead to a similar end result, namely a narrowed vascular lumen.[224]

2. Medial Changes and Development of Longitudinal Muscle Columns

Quantitative morphometric studies have demonstrated the crucial role played by smooth muscle cells of the media in the adaptation of the vessel wall to increased intraluminal pressure.[225-228] Following surgical constriction, for instance, more than 50% of the wall thickening can be attributed to medial smooth muscle cell proliferation. The increase in arterial wall thickening appeared in all experimental models directly attributable to this proliferation and correlated with the increase in medial tension.[229]

The total tension on the coronary arterial wall, estimated at 100 mmHg, was found to be 2.03×10^4 dyn/cm^2 and the medial stress to be 74.5×10^4 dyn/cm^2.[133] Theoretically, this tension seems to be adequate to stimulate medial growth and this can be demonstrated in many arterial beds and in the coronary arteries of laboratory animals. A comparative study on coronary and fixed renal arteries while distended at physiological pressure in ten mammalian species showed that the number of smooth muscle cells and the thickness of the medial layer was augmented with average tension. This augmentation was found to be relatively constant regardless of species, and the estimated tension per layer shows more or less similar values in renal and coronary arteries.[230]

We were often deeply impressed by the poor development of the medial layer in many apparently normal proximal and intermediate segments of the major coronary arteries. This was also observed by other pathologists and the slow increase in the number of circularly oriented rows of smooth muscle cells of the media appeared as a peculiar feature of human coronary arteries. Moreover, a decrease in the medial thickness was frequently recorded, particularly in regions where longitudinal muscle columns made their appearance.[231]

The inadequate coronary medial growth during infancy, childhood, and adolescence,

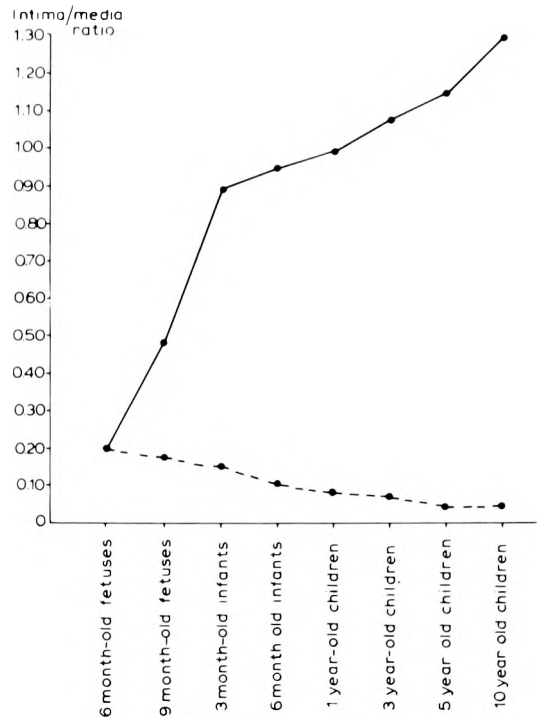


FIGURE 12. Age-related evolution of the intima/media thickness ratio in the proximal segment of the left anterior descending coronary artery (solid line) and a renal artery (dotted line) of similar size. Mean values obtained from five selected cases in each age group. (From Velican, C. and Velican, D., *Atherosclerosis*, 33, 191, 1979. With permission.)

reflected in a reduced capability to add new circularly oriented rows of smooth muscle cells to those preexisting in fetuses and neonates, is difficult to explain and has no correspondence in experimental models. It is also difficult to explain in the light of observations made on other arteries of muscular type and similar size. We showed, for instance, that in the basilar, anterior cerebral, vertebral, renal, hepatic, and bronchial arteries, intimal connective tissue does not develop during fetal life, infancy, and childhood. Consequently, the age-dependent increase in the wall thickness is the result of a progressive agumentation in the number of circularly oriented rows of smooth muscle cells of the media. This leads to a slow decrease of the intima/media ratio with age (Figure 12).[232] In the coronary arteries a different pattern of growth and remodeling was found, as compared to other organ arteries; it is characterized by the very rapid development of a diffuse intimal thickening and slow augmentation of medial thickness. Therefore, the intima/media ratio increases progressively from fetuses to infants and from infants to children. This inability of the coronary media to adapt itself to local hemodynamic conditions has also been clearly recorded in the left anterior descending artery and left circumflex artery during the period in which, after birth, a shift takes place in the coronary blood flow from right ventricular predominance to left ventricular predominance.

Likewise, a study carried out in our laboratory on coronary and renal arteries revealed that in young adults the media of the renal arteries is as thick as the media and the intima of a coronary artery of a similar size and wall thickness (Figure 13). The media represents 80 to 85% of the total wall thickness in the renal artery and only 25 to 30% in the coronary artery.[232] Similar significant differences were detected between coronary and other organ arteries of muscular type and similar size. This inability of the coronary media to adapt

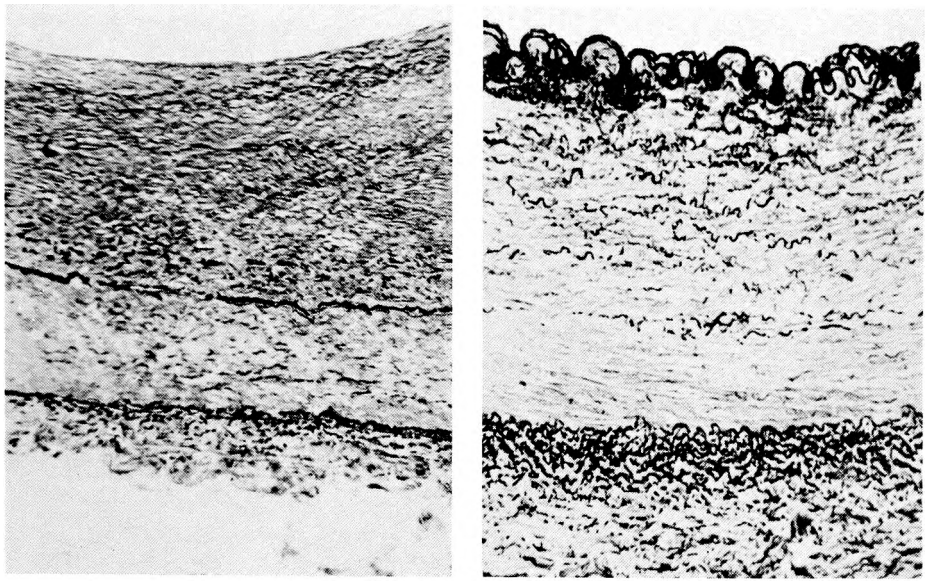


FIGURE 13. Left anterior descending coronary artery (left) and renal artery (right) of a 28-year-old male young adult, both vessels with a size of 3 mm. The media of the renal artery is as thick as the media and the intima of the coronary artery. (Resorcin fuchsin-alcian blue. Magnification × 120.)

itself to local hemodynamic conditions becomes more visible in mature adults who may show an involution or atrophy of the medial layer. It was considered by some investigators as an age-related change,[233] and was recorded even in subjects with cardiac hypertrophy.[234]

This insignificant growth of the coronary medial layer contrasts with the significant development of longitudinal muscle columns. In the basilar, anterior cerebral, vertebral, renal, and hepatic arteries, longitudinal muscle columns can be visualized only by chance. On the contrary, in the coronary and bronchial arteries, longitudinal muscle columns appear as an important and more or less constant component of the vessel wall microarchitecture. Even in full-term fetuses agglomeration of longitudinal muscle columns could often be seen in our material, particularly around the aortic ostia of both coronary arteries and in the paraostial segment of the left main coronary artery and right coronary artery (Figure 14). These longitudinal muscle columns then develop in the proximal and intermediate segments of the major coronary arteries as an age-related change and we were able to record a considerable agglomeration of such structures starting from childhood in the proximal segment of the first diagonal branch and of the left and right marginal branches (Figure 15). This prevalence of longitudinal muscle columns was usually associated with the absence of intimal thickening or with a late and poor development of an intimal layer (Figure 16).

The occurrence of longitudinal muscle columns indicates an added ability of the coronary arterial wall to contract in the direction of blood flow and hence an additional mechanism to regulate blood flow. The longitudinal arrangement of smooth muscle cells may counteract abnormal longitudinal stretch of the wall, particularly in areas with disintegrated internal elastic membrane. This was observed as early as the third postnatal month: longitudinal muscle columns in both the intima and the media contributing to the formation of an intermediary sublayer which occupies the position of the internal elastic membrane. It acquires a particular development in the left main coronary artery and can be clearly delineated even in mature adults (Figure 17). In cases with the absence of left main coronary artery and the left anterior descending artery arising directly from the aorta, the proximal segment of the intermediary sublayer shows a considerable development of longitudinal muscle columns associated with a poor intimal layer (Figure 18).

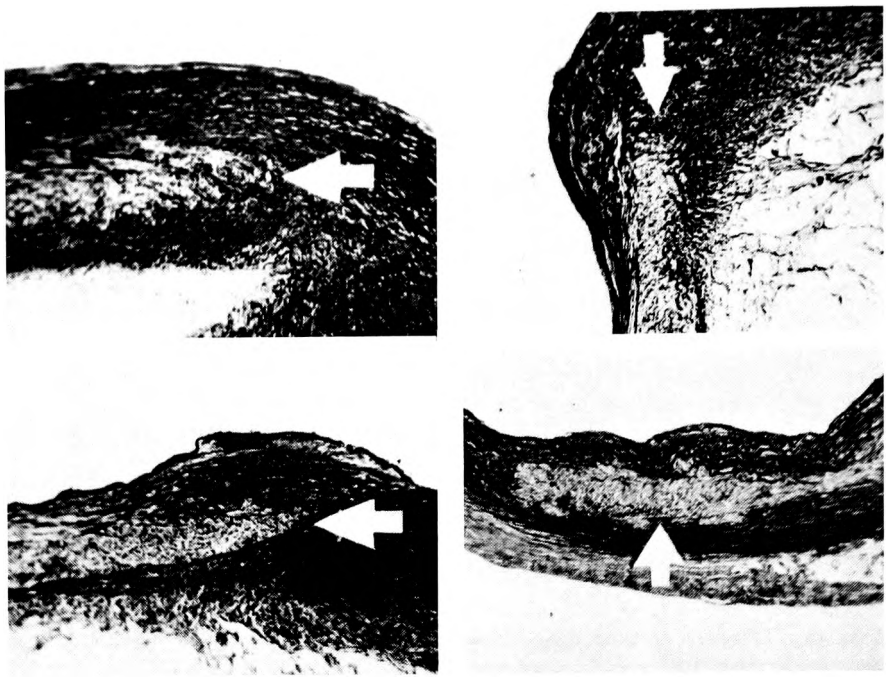


FIGURE 14. Full-term fetuses. Aortic branch sites of the left main coronary artery (left) and right coronary artery (right), showing important agglomerations of longitudinal muscle columns between the elastic laminae (arrow) of the aortic wall. (Resorcin fuchsin-alcian blue. Magnification × 80.)

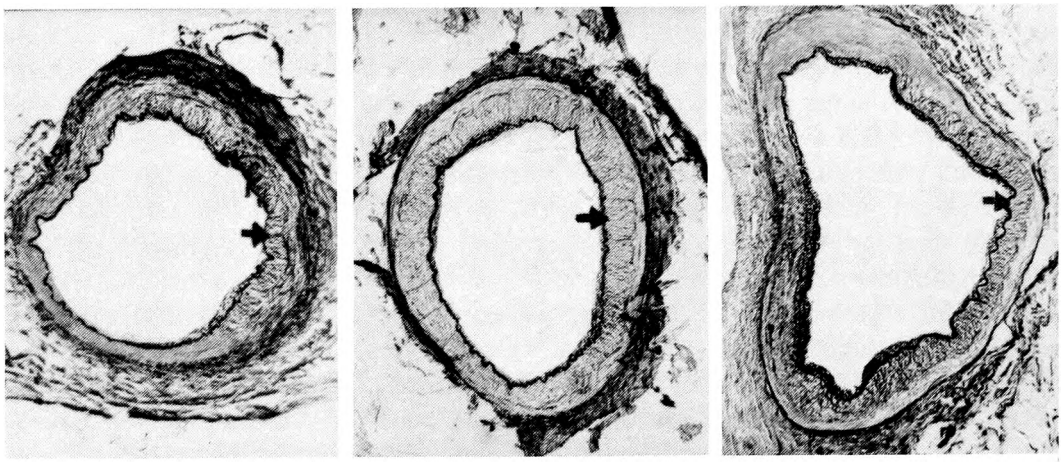


FIGURE 15. Prevalence of longitudinal muscle columns (arrow) in the microarchitecture of the right marginal artery (left), left marginal artery (middle), and first diagonal artery (right). (Resorcin fuchsin-alcian blue. Original magnification × 80.)

Beyond the limited number of investigations that have provided data from human arteries obtained at the time of surgery, most of our understanding of the cellular makeup of those longitudinal muscle columns is derived from specimens obtained at autopsy. They were fixed in formalin or other solutions, embedded in paraffin, and stained with dyes classically used for light microscopic examination. Specimens prepared in this manner provide relatively little information concerning the cells included in these structures. When this information could be obtained, some of these cells appeared to be similar to those of the cardiac conduction system.

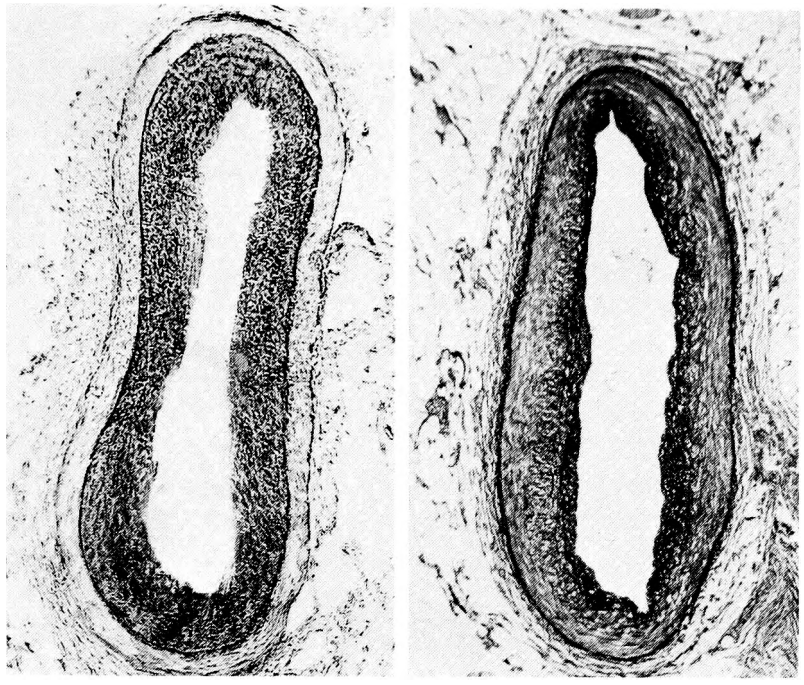


FIGURE 16. The proximal segment of the left anterior descending artery cross sectioned in two similar unbranched sites in two male juveniles, 11 years old. (Left) The vessel wall includes a media without agglomerations of longitudinal muscle columns, a well-developed and preserved internal elastic membrane, and a very thick intima. (Right) The vessel wall includes a media with many longitudinal muscle columns developed at the media-intima frontier; the internal elastic membrane cannot be visualized and the intima is very thin. (Resorcin fuchsin-alcian blue. Magnification × 80.)

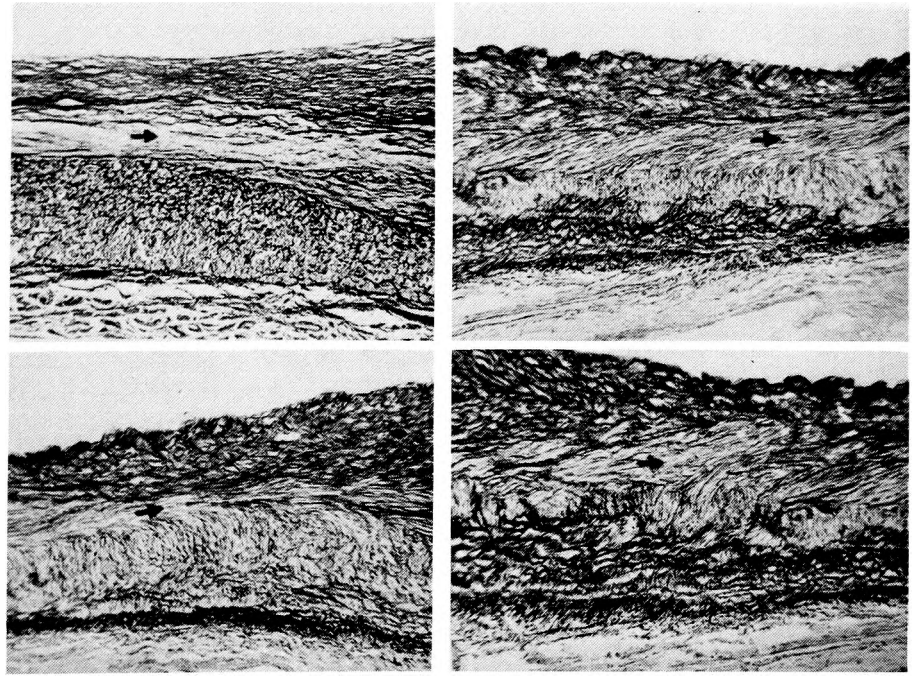


FIGURE 17. Left main coronary artery of four male mature adults, at 0.5 cm from the aortic ostium. Longitudinal muscle columns (arrow) are present as an intermediate sublayer between the media and the intima. (Resorcin fuchsin-alcian blue. Magnification × 120.)

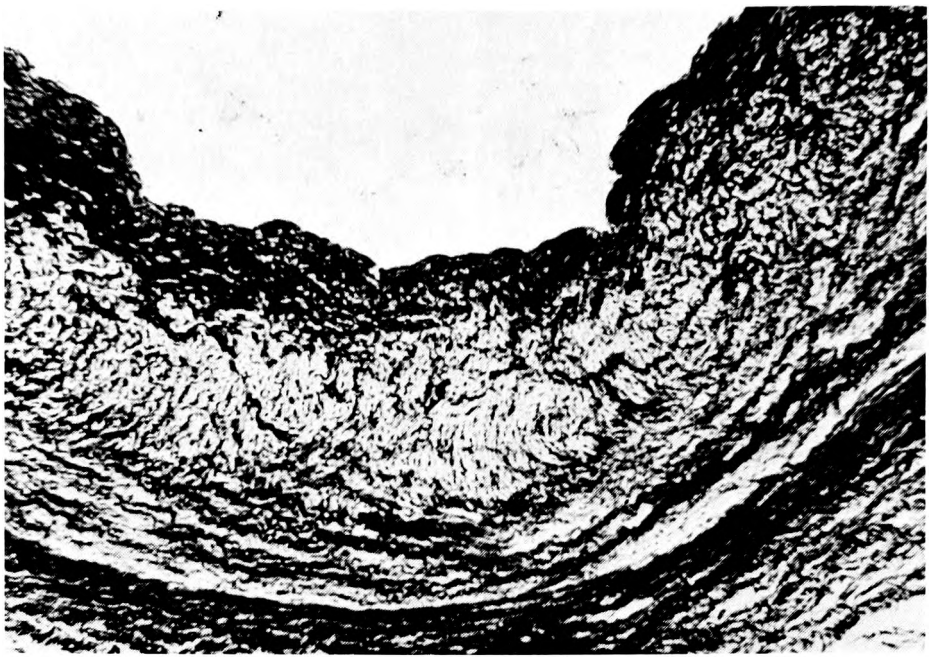


FIGURE 18. Proximal segment of a left anterior descending artery which arises directly from the aorta, cross-sectioned at 0.5 cm from the aortic ostium. Prevalence of longitudinal muscle columns in the vessel wall microarchitecture, associated with a poor development of the intimal layer. (Resorcin fuchsin-alcian blue. Magnification × 220.)

The onset of longitudinal muscle columns in the branches of the bronchial artery was attributed to the rhythmic stretching of the vessels during respiration. This was supported by the results of certain experiments in which the onset of longitudinal muscle columns in the wall of the mesenteric arteries was demonstrated when those vessels were connected to the diaphragm and stretched intermittently with the movements of the diaphragm.[235] In a totally different experimental model, longitudinal muscle columns were formed in the mesenteric artery subsequent to insertion of a ligature through the mesentery.[236]

The musculo-elastic layer in which these longitudinal muscle columns prevail appears prominently in ethnic groups with a high incidence of coronary heart disease, such as Ashkenazy Jews, or the population of eastern Finland. On the other hand, the development of the musculo-elastic layer appears negligible in ethnic groups with a low prevalence and incidence of myocardial clinical manifestations induced by coronary atherosclerosis.[237-240]

A suggestion has been made that this musculo-elastic layer plays a major role in the onset of early atherosclerotic plaques and may be considered a precursor of these early lesions.[241] It seems that the cellular composition of this layer changes over the years and that collagen fibers also become more numerous. This would indicate that a smooth muscle cell proliferation progresses to a fibrotic lesion. According to this view, it is possible to visualize transitional stages between a physiologic adaptation occurring as a musculo-elastic layer and an atherosclerotic plaque.

By means of serial sections and photographic reconstructions we showed that certain longitudinal muscle columns acquire a particular microarchitecture at the level of the coronary arterial branch mouth (Figure 19). The deepest group of longitudinal muscle columns crisscrosses with the opposite similar bundles proximal to the branch mouth and run half of the respective ostium to insert itself on the vessel adventitia. The intermediate group of longitudinal muscle columns also crisscross with the neighboring bundles proximal to the ostium and then continue longitudinally. Finally, the superficial group run in a longitudinal direction right to the branch mouth where each column encircles half of the ostium and then continues

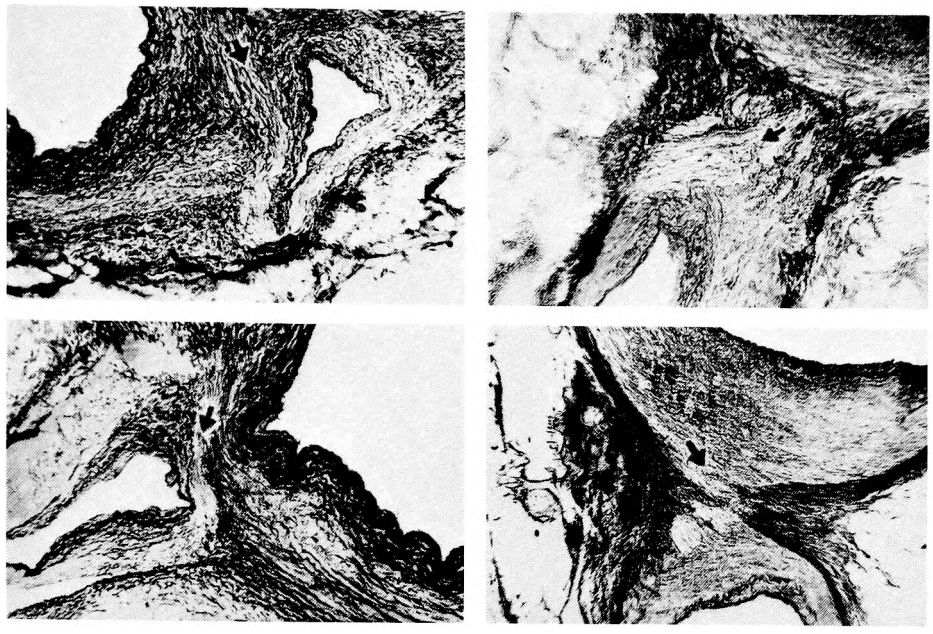
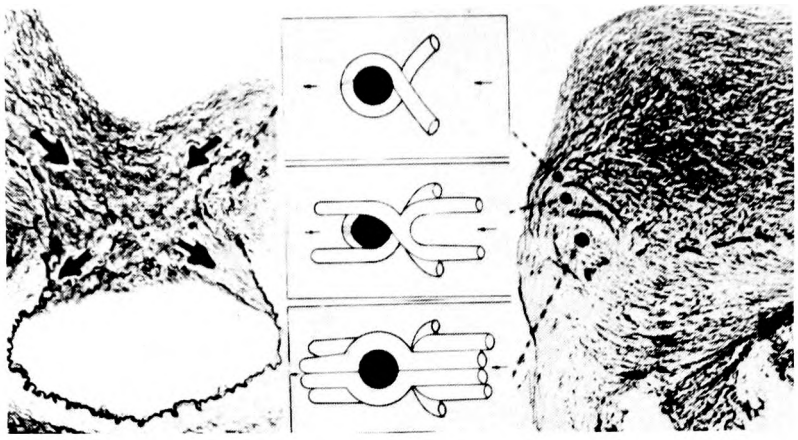


FIGURE 19. Serially cut sections at branch sites of the first septal vessel (upper left), left marginal vessel (bottom, left), right marginal vessel (upper right), and first diagonal vessel (bottom, right). Constant presence of longitudinal muscle columns which criss-cross or encircle the branch mouth (arrow). (Resorcin fuchsin-alcian blue. Magnification × 80.)

parallel to the artery lumen (Figure 20A).[242] A sphincteric microarchitecture was revealed in isolated cases at the branching point of the sinus node and atrioventricular node arteries, of the left and right marginal branches, and of the first and second diagonal branches. The method used is time-consuming and complicated to demonstrate the prevalence of this sphincteric microarchitecture as related to age, race, sex, anatomical branching pattern, blood pressure levels, etc. Nevertheless, its presence could indicate the existence of a mechanism able to produce a significant reduction in the branch mouth diameter, as suggested in Figure 20B. A sphincteric-like microarchitecture was also revealed surrounding the aortic ostium of the right coronary artery.[115] A large smooth muscle mass, differing from the aortic tissue, is involved in this particular microarchitecture. The right coronary artery may have either a circumferential muscle at its orifice or a thick media composed of short sequential segments of muscle, analogous to a connected series of sphincteric muscles, all of which would characterize a system capable of developing high resistance. This sphincteric-like microarchitecture could not be detected at the aortic ostium of the left main coronary artery, suggesting a low resistance system.[115]

An additional peculiar microarchitecture of longitudinal muscle columns of human coronary arteries revealed by us are the hyperplastic muscle columns which encroach upon the branch mouth.[243] It was detected in one out of every five unselected cases and prevailed at the branching points of the first septal and first diagonal vessels. In certain cases these hyperplastic muscle columns may occupy two thirds of the coronary wall thickness. This peculiar microarchitecture was present three times more frequently in patients who died of myocardial infarction or sudden cardiac death than in subjects who died of noncardiac causes. The existence of a relationship was suggested between the presence of areas of myocardial necrosis and of hyperplastic muscle columns encroaching upon some branch mouths.[243] Likewise, a relationship was suggested by our material between (1) the presence of hyperplastic muscle columns encroaching upon the branch mouths of the sinus node and atrioventricular node arteries and (2) the electrical instability of the heart, particularly ventricular fibrillation leading to sudden cardiac death (Figure 21).



A

B

FIGURE 20. (A) Camera lucida drawings from a branching point with a sphincteric microarchitecture. For details, see text. (B) Suggested reduction in the luminal diameter of the branch mouth induced by a possible contraction of the sphincteric microarchitecture. (Resorcin fuchsin-alcian blue. Magnification × 220.)

3. Intimal Thickening and Occurrence of Intercalated Vascular Segments

Figure 22 shows a progressive and significant increase in the relative thickness of the intimal layer of the coronary arterial wall during phylogenetic evolution, with two peaks: (1) the passage from poikylethermy to homeothermy (2) the occurrence of human beings.[244] Only in human beings does intima become the thickest layer of the coronary arterial wall, and the relative thickness of the coronary intima in children is considerably greater than that recorded in adult animals. The capacity of medial smooth muscle cells to migrate in the subendothelial area, to give rise to important amounts of ground substance and fibers and to proliferate, can also be associated with both homeothermy and occurrence of human beings. Figure 23 suggests the relationship between the relative thickness of the coronary intima and the susceptibility to atherosclerotic involvement during phylogenetic evolution.[244] In line with these findings is the absence of spontaneous atherosclerosis in animals without intimal thickening and the onset of myocardial clinical manifestations induced by coronary atherosclerosis only in men.

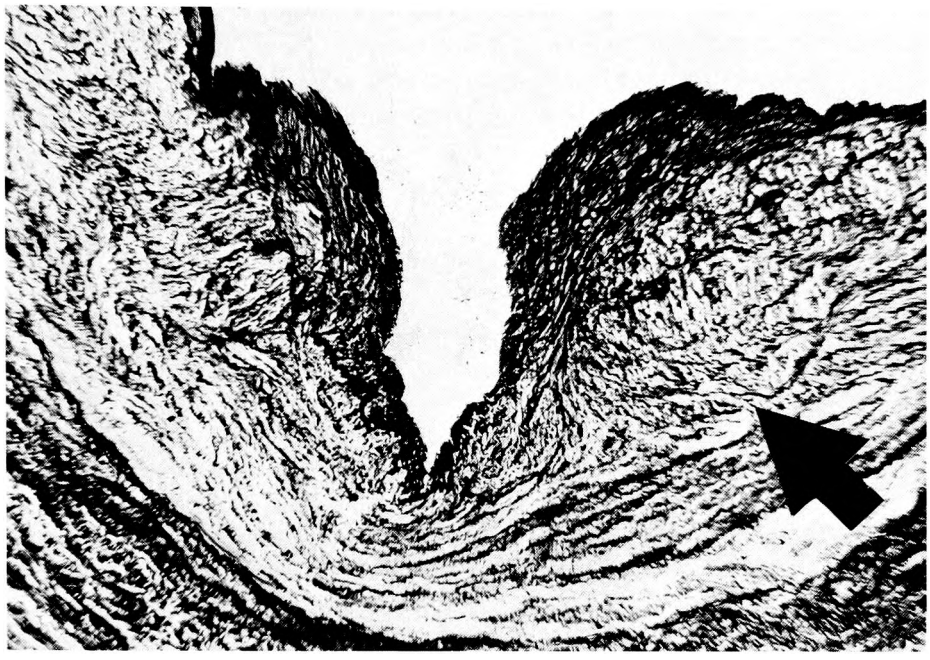


FIGURE 21. A 58-year-old man; clinical diagnosis: sudden cardiac death with ventricular fibrillation resistant to antiarrhythmic therapy. Absence of severe stenotic plaques in the whole coronary arterial bed. The left anterior descending artery exhibited an important accumulation of hyperplastic muscle columns (arrow) which encroached upon the branch mouth at the point of origin of the first septal vessel. (Resorcin fuchsin-alcian blue. Magnification × 220.)

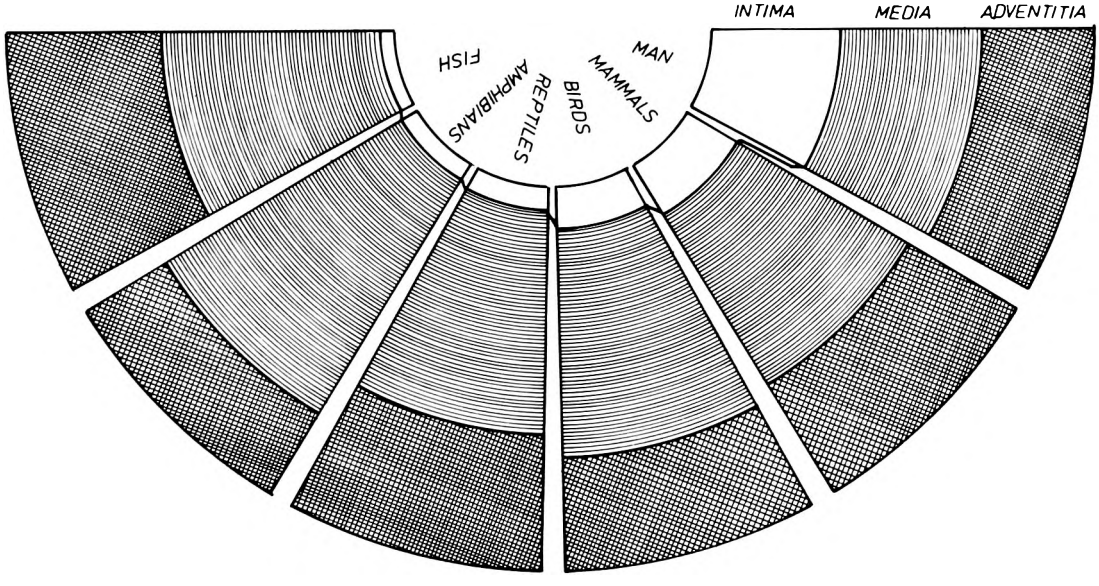


FIGURE 22. Suggested relationship between the phylogenetic evolution and the relative thickness of the coronary artery intima. (From Cucu, Fl., *Rev. Roum. Med., Med. Int.*, 24, 163, 1986. With permission.)

In our material, the intima/media ratio in the 7-month-old fetuses was 0.19 in the left anterior descending artery, 0.17 in the left circumflex artery, and 0.28 in the right coronary artery. This ratio demonstrates a media considerably thicker than the intima. In 9-month-old fetuses it augmented to 0.46, 0.47, and 0.37; in 6-month-old infants to 0.88, 0.90, and 0.60; and in 1-year-old infants to 1.05, 1.0, and 0.96.[232] This would indicate that in the

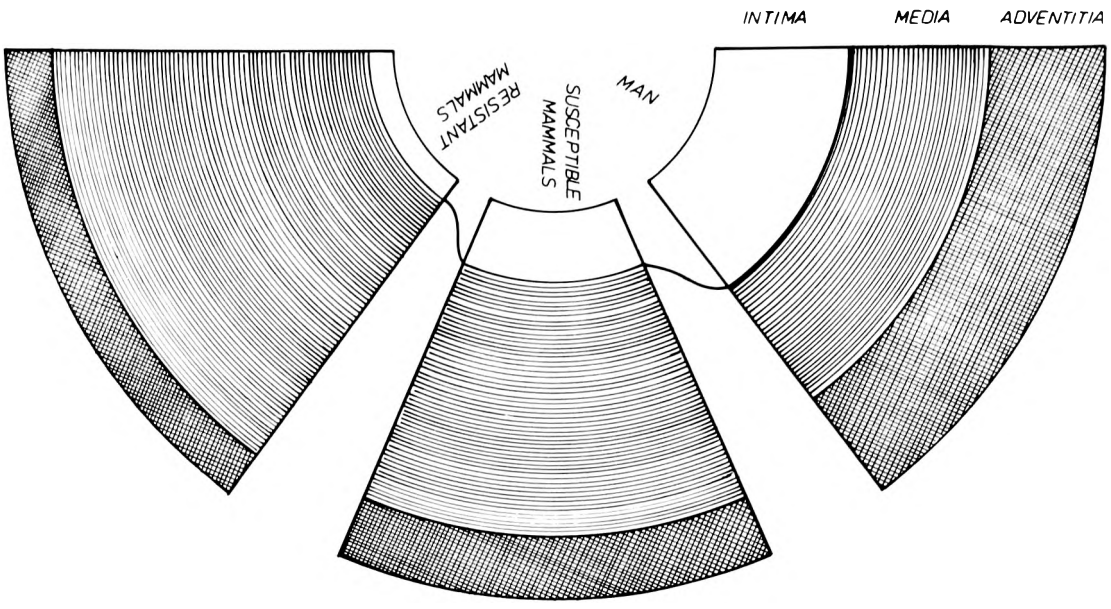


FIGURE 23. Suggested relationship between the relative thickness of the coronary intima and susceptibility to atherosclerosis. (From Cucu, Fl., *Rev. Roum. Med., Med. Int.*, 24, 163, 1986. With permission.)

proximal segment of the major coronary arteries (except the left main coronary artery) intima very rapidly becomes as thick as the underlying media and this could be demonstrated even in 1-year-old infants. During childhood, the intimal layer of the proximal segment of the left anterior descending artery, the proximal segment of the left circumflex artery, and sometimes the intermediate segment of the right coronary artery appears thicker than the underlying medial layer. A particular observation is this centrifugal extension of a very thick intima in the left anterior descending artery and left circumflex artery, compared to the centripetal extension recorded in the right coronary artery. Intimal thickness surpassed media thickness in our material in the left anterior descending artery at:

2 cm from its point of origin in the age group 6 to 10 years old
3 cm in the age group 11 to 15 years old
4 cm in the age group 21 to 25 years old
5 cm in the age group 26 to 30 years old

In the right coronary artery, intima thickness surpassed media thickness in an opposite direction:

5 cm from its point of origin in the age group 6 to 10 years old
4 cm in the age group 11 to 15 years old
3 cm in the age group 16 to 20 years old
0.5 cm in the age group 31 to 35 years old

The most rapid development of the intimal layer was recorded in children and juveniles aged 11 to 15 years. In hospitalized children we also revealed a very thick intima in some coronary branch vessels, particularly those supplying the sinus and atrioventricular nodes. These particular aspects could not be detected in nonhospitalized children and was related to the effect of their terminal disease.[245]

In human coronary arteries, beginning at adolescence, the intima usually becomes the main layer of the vascular wall. This contrasts with other organ arteries of muscular type

and similar size in which a thickened intima is absent during childhood and even during adolescence and early adulthood. Similar conclusions resulted from a comparative study between the coronary artery and the internal mammary artery.[245] After the age of 3 years all coronary vessels showed intimal thickening, whereas in the internal mammary artery this layer remained insignificant until the fifth decade of life. This particular tendency of the major coronary arteries to develop a very early and very thick intimal layer was emphasized in many other investigations.[246-248]

All these results demonstrate that the coronary arteries are submitted to particular genetic influences and particular hemodynamic stresses than any other artery of comparable size. There are also important variations in the degree and extension of intimal thickening from one case to the other. The unfortunate individuals who develop a very thick coronary intima starting from childhood and adolescence are considered prone to early "coronary artery insufficiency".[246] Others have a slow rate of intimal thickening and consequently are likely to live to a much greater age before myocardial clinical manifestations become critical for survival.

Our studies also revealed that in similar topographic sites of the major coronary arteries and of the main branch vessels intimal thickening developed 5 to 15 years earlier in subject with, then in subjects without, some minor deviations from the common type of distribution of the coronary arteries.[249,250] In 10% of unselected cases the left circumflex artery showed considerable development coexisting with a short and narrow right coronary artery. In such individuals we found intimal thickening in the proximal segment of the left circumflex artery about 10 years earlier than in cases without this minor deviation from the common type of distribution of the coronary arteries. In 9% of cases the posterior descending artery showed a considerable development coexisting with one or two short and narrow anterior descending arteries. In such cases, intimal thickening was seen in the posterior descending artery in the age group 6 to 10 years old, as in a major coronary artery, compared to the 21 to 25 years old age group in subjects with the common type of distribution of the coronary arteries. Other examples in this respect were presented in a recent study.[251] It demonstrates that in mature adults 46 to 50 years old intimal thickening in the first septal vessel and in the vessels supplying the conduction system was detected in one out of every three to four cases. This would indicate that more than $^1/_4$ of individuals considered to be in the preclinical stage of coronary heart disease have intimal thickening in the vessels usually not removed for light microscopic examination. The proportion of subjects 46 to 50 years old with intimal thickening in the main coronary branch vessels increased to more than 50% if the first diagonal, posterior descending, left, and right marginals were also examined by light microscopy. Our results show that the views which assume that in the coronary arterial tree intimal thickening develops only during childhood are unrealistic simplifications. Each segment of the coronary arterial tree has its own period of life in which intimal thickening develops, related to sex and anatomical branching pattern, as well as to the presence of an associated terminal disease.[249,250] Whereas in the major coronary arteries the thickened intima frequently includes a basal musculo-elastic sublayer, an intermediate elastic hyperplastic sublayer and a subendothelial ground substance-rich sublayer, in the coronary branch vessels less than 2 mm in external diameter this stratification progressively disappears. It is replaced by an apparently fortuitous accumulation of smooth muscle cells, ground substance, and fibers between endothelium and media without a special arrangement.

The reason for which a thickened intima begins to develop starting from a given site of an artery cross-section (Figure 24) or acquires a stratified character and becomes thicker than the underlying media is not clear. Additional data on this subject are presented in Chapter 4. It is likewise not clear why in cases of similar age, sex, anatomical branching pattern, and cause of death, different degrees of intimal thickness and various aspects of intimal microarchitecture can be seen in similar topographic sites. Certain of these static

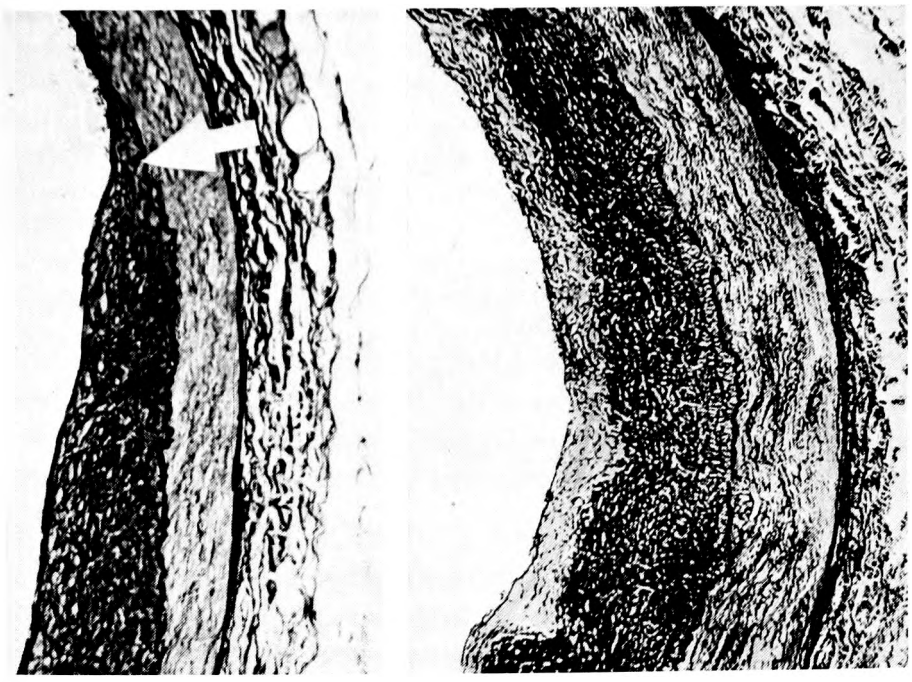


FIGURE 24. (Left) Sudden passage (arrow) from the absence of a thickened intima to a very thick intima. Intermediate segment of the right coronary artery of a 28-year-old female. (Right) Stratified aspect of the thickened intima, including a basal elastic hyperplastic sublayer and a superficial ground-substance rich sublayer. Proximal segment of the left anterior descending artery of a 25-year-old male. (Resorcin fuchsin-alcian blue. Magnification × 120.)

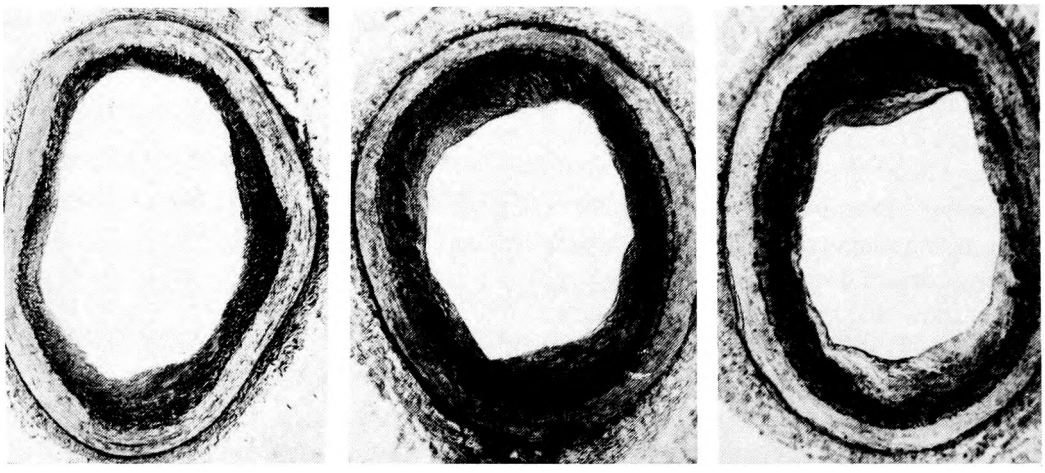


FIGURE 25. Three cross-sections of a similar topographic site of the left circumflex artery from three subjects aged 18, 26, and 32 years. The succession of changes from left to right might suggest a progressive passage from an apparently nonatherogenic to an atherogenic thickened intima. (Resorcin fuchsin-alcian blue. Magnification × 80.)

aspects might suggest a passage from a nonatherogenic to an atherogenic thickened intima (Figure 25).

The influence of the degree of coronary intimal thickening of vessel wall metabolism is speculative. The thickened coronary intima is an avascular layer under the influence of the passage of plasma oxygen and metabolites across the respective wall. This dependence on

the transport of plasma components is determined by the lack of capillaries for direct blood supply. Consequently, local variations in perfusion pressure, as well as in the degree of intimal thickness, could have important effects on the coronary intima metabolism. There seems to exist a "critical depth" allowing a minimum adequate oxygen supply for intimal smooth muscle cells. When this critical depth is surpassed, a prolonged intimal hypoxia appears, leading to the impairment of smooth muscle cell metabolism and to the occurrence of cell necrosis. Minimal changes in macromolecular organization, composition, and aggregation of the intimal ground substance, collagen, and elastic fibers might be of significance for an adequate metabolism of smooth muscle cells. The adaptation to these special requirements results in a considerable metabolic flexibility, which allows the smooth muscle cells to function in relatively anaerobic conditions. A particular region seems to exist in each area of the thickened intima where filtration pressure from the lumen equals the capillary pressure of the vasa vasorum. The moving frontiers of these regions are continuously influenced by (1) intraluminal pressure, (2) additional hemodynamic and mechanical stresses, (3) functional state and integrity of the endothelial cells, (4) thickness of the intima, (5) amount of fibrillar component of the intimal connective tissue, (6) fenestrations of elastic laminae, and (7) pressure within the vasa vasorum. Gradual obstruction of adventitial lymphatics could lead to inadequate clearance of transintimal filtrate from the avascular zone and therefore could promote lipid and fibrin deposition. Moreover, severe obstructions of adventitial lymphatics may be followed by intimal and medial ischemia and necrosis.

The degree of intimal thickening is important not only for the above-mentioned possible alteration produced in the metabolism of coronary arterial wall, but also for the significant luminal narrowing which may result. An intima/media ratio of 2.0 to 3.0 was associated in our material with an approximately 50% reduction in the luminal diameter; when intimal/media ratio surpasses 3.0, the luminal diameter may be reduced up to 75%.[251] An intimal layer exhibiting this obstructive character was mainly recorded at 5 to 8 cm from the point of origin of the right coronary artery, at 2 to 4 cm from the point of origin of the left anterior descending artery, at 2 to 3 cm from the point of origin of the left circumflex artery, and in the proximal segment of the vessels supplying the sinus node and the atrioventricular node.

The term "intimal thickening" is used in the available literature to present a multitude of arterial wall responses to physiological and pathological stimuli. An eloquent example is the use of a similar term to describe an age-dependent change in the coronary arteries of children and a change of a peculiar character occurring in aorto-coronary bypass grafts or in other particular hemodynamic conditions.[252-255] The high degree of correlation detected in our material between the coronary anatomical branching pattern and coronary intimal thickening adds new, indirect observations which suggest the prevalent role of hemodynamic stresses. This prevalent role has been clearly demonstrated in the vessels of the hypervolemic partner of a pair of twins affected with placental transfusion syndrome.[256]

Strongly related to the development of a thickened intima in the coronary arterial bed is the occurrence in fetuses, infants, and children of a particular microarchitecture, tentatively termed by us "intercalated vascular segment".[257] We proposed this term to designate a connection between a major coronary artery and a main branch vessel occurring as a transintimal cylindrical conduit, lined by endothelium and encased by the intimal connective tissue of the major coronary arteries (Figure 26). This cylindrical conduit lacks a muscular media, an adventitia, and even an internal elastic membrane and seems to offer a reduced mechanical resistance. It could be presumed that it is easily narrowed or obstructed by pathological changes occurring in the neighboring tissue. Likewise, it is probably not capable of vasomotion (active vasoconstriction and dilation) due to the absence of a muscular media. Therefore, it cannot respond to catecholamines, nervous influences, or to drugs. The intercalated vascular segment seems to act as physiological bypass developing only passive mechanical properties.

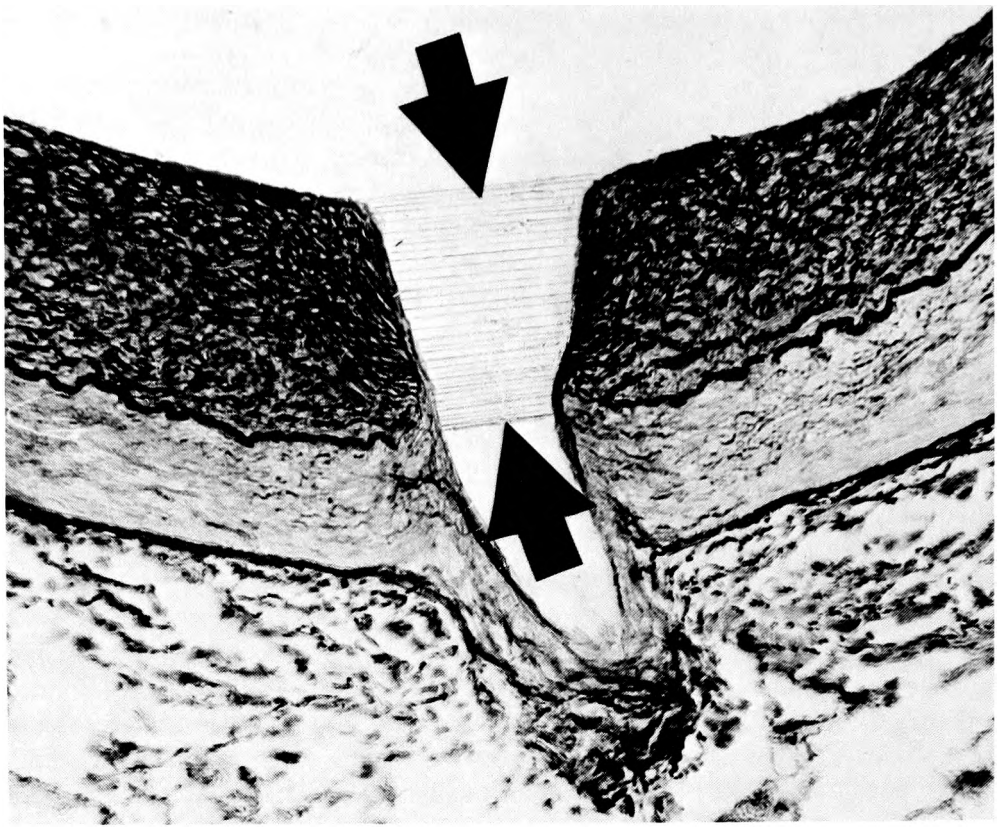


FIGURE 26. Point of origin of the first septal branch of the left anterior descending artery in an 18-year-old male subject. The *intercalated vascular segment* (hatched area) can be seen as a structural accommodation of the initial portion of the coronary branch vessel. It appears as a transintimal cylindrical conduit, lacking a muscular media, an adventitia, and an internal elastic membrane, being encased by the thickened intima (black) of the major coronary artery. The hatched area shows that the length of the intercalated vascular segment represents the thickness of the intimal layer of the major coronary artery. (From Velican, D. and Velican, C., *Atherosclerosis*, 60, 237, 1986. With permission.)

The branch sites of the coronary arterial tree where intercalated vascular segments are usually present are indicated in Figure 27.

The histoarchitecture of a similar coronary branching site with and without an intercalated vascular segment is presented in Figure 28.

In an attempt to reveal the onset and development of this peculiar vascular structure, selected branching sites were grouped according to age, sex, and anatomical branching pattern. With this method we observed that as the intimal layer of a major coronary artery became thicker and thicker, an accommodation of the connection between this major coronary artery and a main branch vessel occurred, leading to the development of a transintimal cylindrical conduit (Figure 29). The medial muscular coat which is absent along the transintimal course of the intercalated vascular segment reappears at its distal end point where the muscular coat of the parent artery continues with the muscular media of the branch vessel. Figures 26 to 28 show that the length of the intercalated vascular segment actually represents the thickness of the intimal layer of the parent artery. The intercalated vascular segment is usually surrounded, in a major coronary artery, by a muscle-elastic sublayer in its basal region, by an elastic hyperplastic sublayer in its intermediate region and by a ground substance-rich sublayer in its subendothelial region (Figure 30).

Chronologically, the earliest intercalated vascular segments were seen at the branching points of the first diagonal and first septal vessels in children 1 to 10 years old (Figure 31).

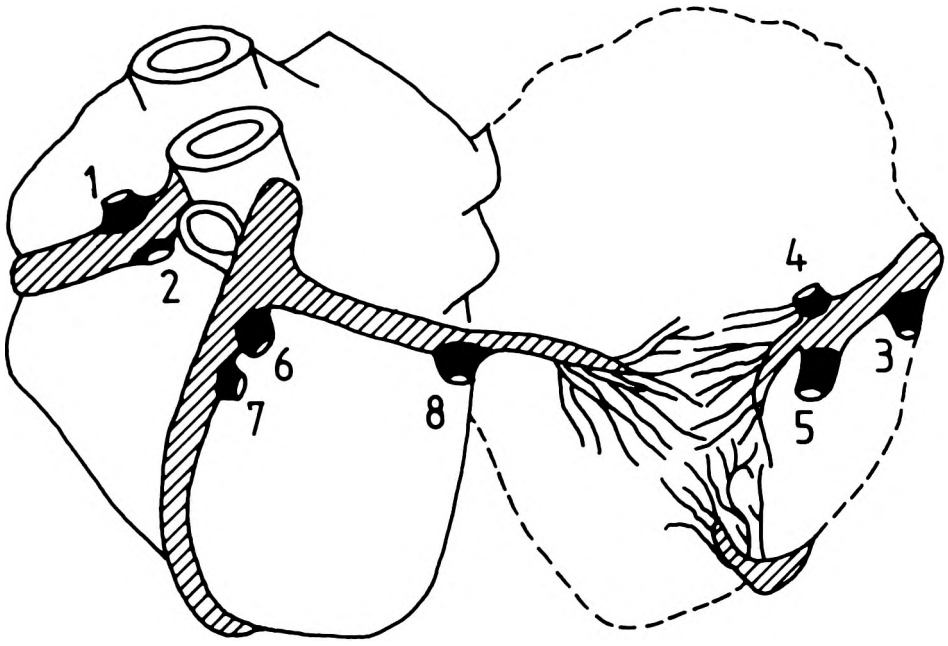


FIGURE 27. Schematic presentation of the coronary arterial tree with branch sites where intercalated vascular segments can be usually detected, namely points of origin of the (1) conus, (2) sinus node, (3) right marginal, (4) atrioventricular node, (5) posterior descending, (6) first diagonal, (7) first septal, and (8) left marginal branches.

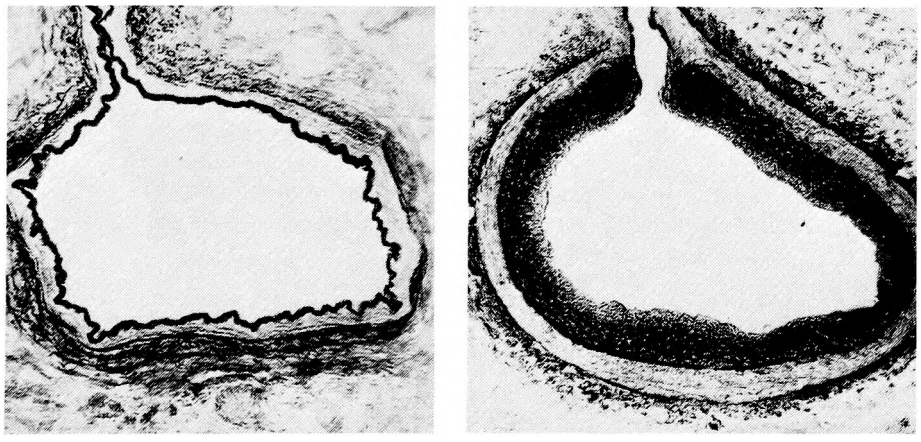


FIGURE 28. Two similar branch sites in two male subjects aged 16 years. (Left) Microarchitecture of a branch site of the left circumflex artery without a thickened intima and implicitly an intercalated vascular segment occurring as the initial portion of the left marginal branch. (Right) Microarchitecture of a branch site of the left circumflex artery with a thickened intima and implicitly with an intercalated vascular segment occurring as the initial portion of the left marginal branch. (From Velican, D. and Velican, C., *Atherosclerosis*, 60, 237, 1986. With permission.)

During the second decade of life, intercalated vascular segments develop at the branch sites of the posterior descending, right marginal, and left marginal vessels. Finally, during the third decade of life, intercalated vascular segments could be visualized at the branch site of the conus vessels, as well as of the vessels supplying the sinus node and the atrioventricular node.

A comparative investigation was carried out in our laboratory on the length and size of intercalated vascular segments of similar branch sites in male subjects 11 to 20 and 41 to

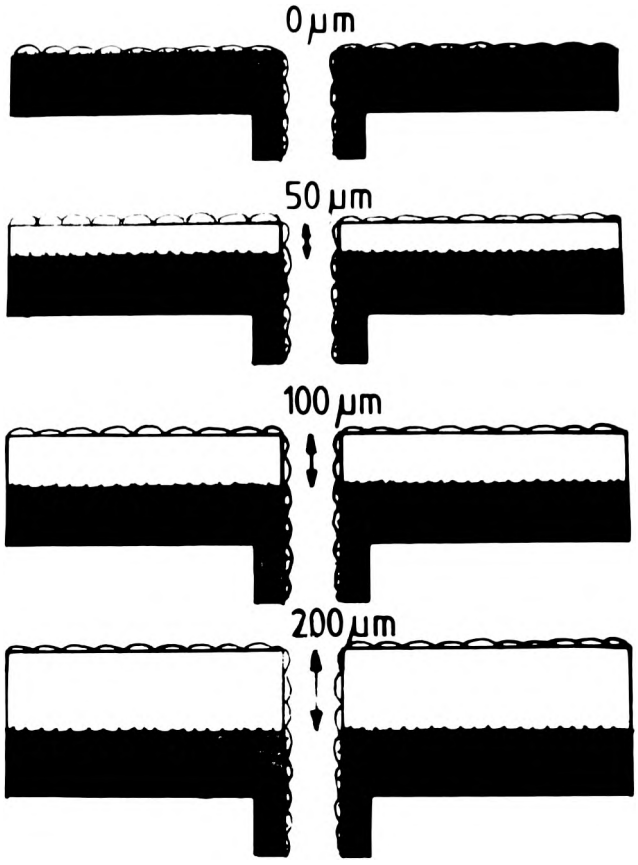


FIGURE 29. Schematic presentation of intercalated vascular segment histogenesis. As the intimal layer becomes progressively thicker (50 to 100 to 200 µm), the intercalated vascular segment becomes longer. This figure also shows that the medial muscular coat (black) which is absent along the transintimal course of the intercalated vascular segment reappears at its distal end; at this point the muscular coat of the parent artery continues with the muscular media of the branch vessel.

50 years old who died in accidents, all with the common type of distribution of the coronary arteries. It demonstrates that, on an age group basis, the length of the intercalated vascular segment of the first septal, first diagonal, posterior descending, left, and right marginal branch vessels increased 4 to 4.6 times during a period of about 30 years. Conversely, the luminal diameter of the intercalated vascular segment near its point of origin decreased 1.51 to 1.76 times with advancing age. Whereas the augmentation in length of the intercalated vascular segment could be clearly correlated with a progressive intimal thickening of the parent vessel, the diminution in the transversal luminal diameter with advancing age seemed to be induced by two types of changes: (1) A concentric fibrous hyperplasia of the intimal connective tissue of the parent vessel which encroached upon the lumen of the intercalated vascular segment; when a severe fibrous concentric hyperplasia was present, the original lumen of the intercalated vascular segment appeared significantly reduced, and the respective segment acquired the light microscopic feature of a stenosed vessel. (2) The second type of change is produced by atherosclerotic plaques developing at branch sites. A reduction in the original luminal diameter of the intercalated vascular segment was recorded whenever a fibromuscular plaque appeared and especially when it progressed to a fibrohyaline one. As a result of both concentric fibrous hyperplasia of the thickened intima and of plaque development, the first narrowed intercalated vascular segments were recorded in an unse-

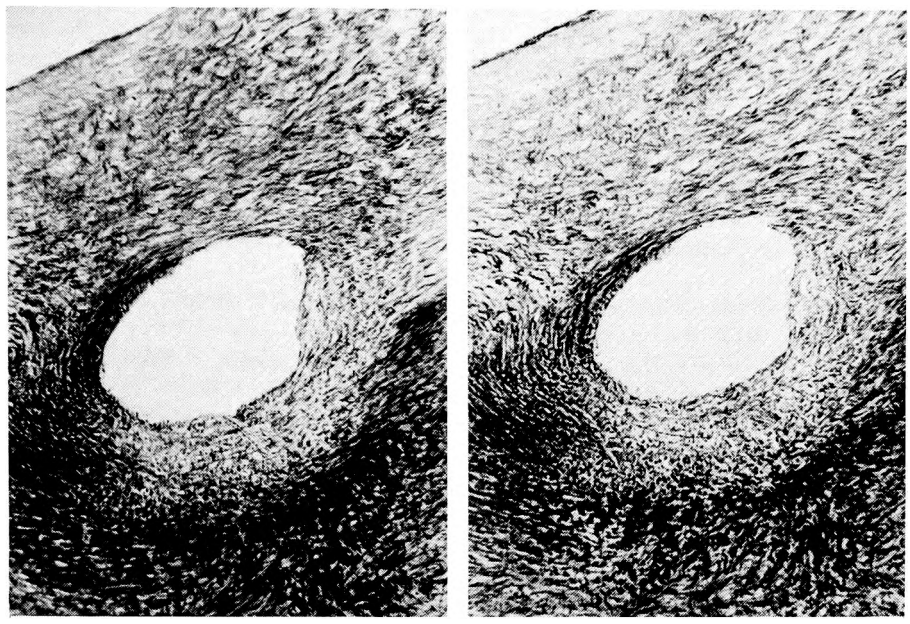


FIGURE 30. Two successive oblique sections revealing the intimal connective tissue of the major coronary artery surrounding the lumen of the intercalated vascular segment. (Resorcin fuchsin-alcian blue. Magnification × 220.)

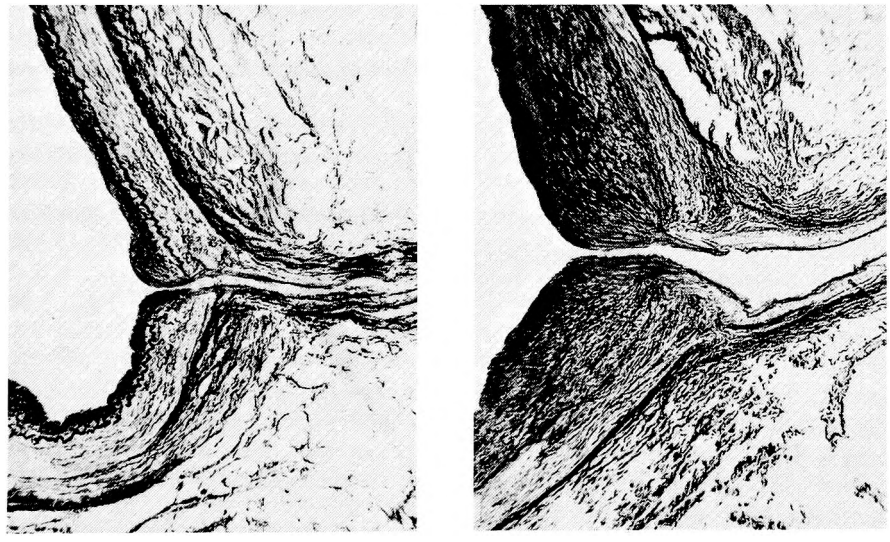


FIGURE 31. (Left) Intercalated vascular segment as seen at the branch site of the first septal vessel in a 1-year-old child. (Right) Similar branch site in an adult aged 32 years. (Resorcin fuchsin-alcian blue. Magnification × 80.)

lected population who died in accidents (male subjects 31 to 40 years old) at the branch site of the first septal and first diagonal vessels.

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Chapter 2

METHODS FOR EVALUATING THE NATURAL HISTORY OF CORONARY ATHEROSCLEROSIS

I. POST-MORTEM STUDIES

A. Coronary Anatomy as Related to Coronary Atherosclerosis

1. General Observations

There are five main avenues of research on the natural history of coronary atherosclerosis:

1. Post-mortem studies
2. Angiographic studies
3. Epidemiologic studies
4. Clinical studies
5. Experimental studies

Of these five avenues, only post-mortem investigations are able to detect and record the succession of stages leading from the early changes to lesions of possible clinical significance. Coronary angiography cannot detect the early lesions, but is very useful for visualizing stenotic atherosclerotic plaques in both asymptomatic and symptomatic cases. Epidemiology is limited to the analysis of risk factors as related to the end stages of the natural history of coronary atherosclerosis, and of the existence of particular environmental conditions, without any possibility of investigating the time-dependent onset and progression of early coronary atherosclerotic lesions. Likewise, clinical data are restricted to the end stages of the natural history of coronary atherosclerosis occurring as angina pectoris, myocardial infarction, or sudden cardiac death. Finally, a variety of experimental models have been developed for the study of the natural history of coronary atherosclerosis, but none of them duplicate the human disease. It is evident that only post-mortem studies can give a more adequate account of: (1) the chronology of arterial wall changes (which appear first and which later); (2) the variety of early lesions; (3) the components which prevail in each type of early and advanced lesions; (4) the succession of stages included in the natural history of coronary atherosclerosis; and (5) the relationships between coronary advanced lesions and myocardial clinical manifestations occurring as coronary heart disease.

These post-mortem studies have also revealed an unexpected close relation between coronary artery anatomy (anatomical branching pattern of the coronary arteries) on the one hand, and atherogenesis, atherosclerotic lesion progression, silent myocardial ischemia, angina pectoris, myocardial infarction, and sudden cardiac death, on the other. The results published by many teams of experienced pathologists, as well as our own investigations, advocate for the existence of the above-mentioned close relation. These results demonstrate that in an age of sophisticated technology and research in depth, there is still the need, not only for a meticulous post-mortem investigation of the major coronary arteries and main branch vessels, but also for a correct evaluation of the anatomical branching pattern of the coronary arteries. Particularly when severe stenotic lesions, associated with myocardial clinical manifestations or with signs of myocardial ischemia, are present in adolescents and young adults, examination of the anatomical branching pattern of the coronary arteries is of major importance for a realistic anatomo-clinical correlation; the younger the case, the more likely might a peculiar anatomical branching pattern be related to myocardial clinical manifestations.

The post-mortem examination of the coronary arterial tree is not a trivial task which must

be performed in a simplified form in only a few minutes, but, on the contrary, requires the investment of an important amount of time and knowledge. An inadvertent technique of examination can overlook lesions of possible clinical significance, can minimize the role of geometric risk factors, or can destroy particular branch vessels, or delicate structures as the sinus node, or an accessory atrioventricular conduction pathway.

We agree with all investigators who assume that the key in a study on the natural history of coronary atherosclerosis is to examine the whole coronary arterial tree (and not only the proximal segments of the major coronary arteries), together with the conduction system, nerves, and ganglia of the heart. This examination requires beginning with the anatomical branching pattern of the coronary arteries. If in 6% of cases (as in our unselected sample of Bucharest), the circumflex artery arises as a branch of the right coronary artery and not as an independent major coronary artery; the intimal surface of the circumflex artery is covered with fatty streaks and raised lesions must be included in that of the right and not of the left coronary arterial system. The investigators who overlook this particular anatomical branching pattern cannot mask an unrealistic starting point with sophisticated mathematical treatments. Moreover, in these cases the right coronary artery is the main vessel supplying the conduction system even if the sinus node and atrioventricular node arteries arise from the circumflex artery. This observation might be of paramount importance for a realistic anatomo-clinical correlation in patients with severe arrhythmias followed by sudden cardiac death.

The pathologist should be routinely armed with knowledge of the origin, course, termination, and mode of the distribution of each major coronary artery and of each main branch vessel. This is also a *sine qua non* condition for all correlations in which post-mortem data are superimposed on those obtained during life by means of coronary angiography, nuclear medicine investigations, echocardiography, computed axial tomography, etc.

This new orientation has as yet very limited access in the field of post-mortem examination and therefore, the bias in pathologic studies inclines toward the differences in age, sex, and race distribution, as well as in the amount of grossly visible lesions. On the other hand, the variation in anatomical branching pattern of the coronary arterial tree is usually overlooked. The possible presence of stenotic or occlusive atherosclerotic plaques in the main coronary branch vessels is also overlooked. This tendency is unrealistic since, for instance, the magnitude of the septal flow to the left ventricle exceeds the right coronary artery flow by 18%.[1] Septal arteries supply 30% of the total left ventricle[2] and 15% of the total myocardium.[3] Particular importance is placed on this vessel in cardiac surgery, reflected by the increasing number of procedures for its direct or indirect grafting. It is thus not possible to adequately understand the natural history of coronary atherosclerosis without macro- and microscopic examination of the atherosclerotic involvement of the main septal vessel. Unfortunately, owning to a strict adherence to oversimplified protocols, such as analysis does not exist in the available literature.

The real significance and scientific value of the extent of the intimal surface of the major coronary arteries involved with raised lesions is questionable if we overlook the possible presence of occlusive plaques: (1) in the proximal segment of the posterior descending artery in patients with posterior myocardial infarction; (2) in the proximal segment of the first diagonal branch in patients with anterior infarction; (3) in the proximal segment of the marginal branch in patients with lateral infarction; (4) in the proximal segment of the first septal vessel in patients with septal infarction; and (5) in the proximal segments of the vessels supplying the sinus node and the atrioventricular node in patients with severe rhythm disturbances and sudden cardiac death.

The view that the coronary arterial tree includes only large vessels, the so-called major coronary arteries and small vessels, is an oversimplified one. Pathologists can often visualize a posterior descending artery similar in size with the right coronary artery, a first or second

diagonal branch similar in size with the left anterior descending artery, and a left marginal branch similar in size to the left circumflex artery.

In essence, one cannot continue to overlook the atherosclerotic involvement of the main branch vessels of the coronary arterial tree and also the fact that anatomic or geometric risk factors may play an important role in atherogenesis, in progression of atherosclerotic lesions, and in the onset of lesions of possible clinical significance.

The existence of these anatomic or geometric risk factors strongly requires more adequate autopsy protocols. How many centimeters should be opened to detect atherosclerotic lesions in a very long right coronary artery measuring more than 12 cm which ends at the crux cordis and in a short right coronary artery measuring less than 5 cm which ends as the right marginal branch? Such different lengths are encountered in one out of every seven individuals and strongly influence the magnitude of the intimal surface covered with atherosclerotic lesions visible on gross inspection. Important differences in length from less than 4 cm to more than 8 cm can be seen in one out of every eight cases concerning the left circumflex artery. In one out of every ten cases the anterior descending artery has a very long course and ascends in the posterior interventricular groove; if only 3 to 4 cm are opened longitudinally, the presence of distal occlusive atherosclerotic plaques can be missed.

The following example (based on the results obtained in our laboratory on an unselected Bucharest population sample, including subjects who died in traffic accidents and with a normal lifestyle before the respective accident) is presented to demonstrate that the simplified autopsy protocols could be an important source of bias; if in 100 male subjects aged 51 to 55 years, gross inspection was limited to the proximal and intermediate segments of the major coronary arteries, 52 subjects showed more than 50% plaque narrowing of the vessel lumen (left main coronary artery, anterior descending, circumflex, and right coronary arteries). If the gross inspection included not only the proximal and intermediate segments of the major coronary arteries, but also the proximal segment of the main branch vessels (posterior descending, first diagonal, first septal, left, and right marginals), the number of cases with more than 50% luminal narrowings augmented from 52 to 61 in the same population sample. This clearly shows that many stenotic or occlusive atherosclerotic plaques are systematically neglected during routine autopsies. To open longitudinally the proximal segment of the major coronary arteries, if these vessels are large enough and readily accessible, requires only a few minutes. To identify all branches of the coronary arterial tree up to 1 mm external diameter and to remove cross-sections from other small branches requires 30 to 60 min; the expense of time is compensated by the richness of information obtained.

These general observations try to explain why in this book particular emphasis has been laid on usually neglected factor: *the anatomy of the coronary arterial bed as related to the natural history of coronary atherosclerosis*. During the last decades, two eminent scientists, Giorgio Baroldi and Thomas N. James have dominated this orientation and Baroldi's assumption[6] might be a motto for the pathologist: "each heart has its own vascular physionomy".

Unfortunately, many recent works continue to present a standardized description of human coronary arteries. This might lead to the unrealistic assumption that all coronary arterial trees would have a similar anatomical branching pattern adequate for comparative measurements of the intimal surface involved with fatty streaks and raised lesions. Since all coronary arteries have a fixed and invariable anatomical branching pattern, they are exposed to similar hemodynamic stresses. Similar hemodynamic stresses in similar anatomical branching patterns are not able to act more intensely as atherogenic agents in some subjects than in others. This erroneous view could explain why body weight, body mass index, various anthropometric measurements, the assessment of sexual maturity, or the fat composition of human milk are considered "precursors" of atherosclerosis and not some particular anatomical branching patterns of the coronary arteries. Our own experience shows that it is not

Table 1
PREVALENCE OF THE COMMON (C) TYPE OF DISTRIBUTION OF THE CORONARY ARTERIES AND OF VARIOUS DEVIATIONS (D) FROM THIS COMMON TYPE IN AN UNSELECTED POPULATION SAMPLE OF BUCHAREST

Age groups (years)	Cases studied		C	D
1—5	T	103	59	44
	M	61	33	28
	F	42	26	16
6—10	T	83	48	35
	M	42	23	19
	F	41	25	16
11—15	T	98	57	41
	M	53	30	23
	F	45	27	18
16—20	T	77	46	31
	M	41	27	14
	F	36	19	17
21—25	T	88	48	40
	M	51	28	23
	F	37	20	17
26—30	T	81	45	36
	M	46	28	18
	F	35	17	18
31—35	T	87	45	42
	M	52	27	25
	F	35	18	17
36—40	T	95	59	36
	M	56	29	27
	F	39	30	9
41—45	T	108	63	45
	M	66	37	29
	F	42	26	16
46—50	T	112	69	43
	M	68	46	22
	F	44	23	21
Total	T	932	539	393
	M	536	312	224
	F	396	227	169

T: total, M: males, F: females.

possible to carry out an adequate study on the natural history of human coronary atherosclerosis if we are not armed with the knowledge of the wide variations in the number, size, topography, and distribution of the coronary arteries (Tables 1 and 2).

2. Left Main Coronary Artery

The left main coronary artery is a major coronary artery occurring as a short vessel (8 to 12 mm) without branches; it terminates by dividing into two other major coronary arteries, the left anterior descending and the left circumflex arteries.

The left main coronary artery supplies an average of 63.8% and the right coronary artery

Table 2
MINOR DEVIATIONS FROM THE COMMON TYPE OF DISTRIBUTION OF THE CORONARY ARTERIES (IN THE DECREASING ORDER OF THEIR FREQUENCY) SUGGESTED BY US TO BE INVOLVED IN THE NATURAL HISTORY OF CORONARY ATHEROSCLEROSIS

Minor atherogenic deviations	Cases %
The right arterial system (right coronary artery, right marginal, posterior descending) showed a considerable development starting from childhood coexisting with a short and narrow circumflex artery which may end as the left marginal branch	12
The left circumflex system (left circumflex, left marginal, posterior descending) showed a considerable development starting from childhood coexisting with a short and narrow right coronary artery which may end as the right marginal branch	10
The right posterior descending artery showed a considerable development which coexisted with one or two short and narrow anterior descending arteries	9
The anterior descending artery showed a considerable development starting from childhood which coexisted with one or two short and narrow posterior descending arteries	7
The left main coronary artery trifurcates, giving rise to a large and long diagonal vessel which duplicates the left anterior descending artery	6.5
One or two of the main branch vessels (posterior descending, first diagonal, first septal, left marginal, right marginal) showed a precocious augmentation of the luminal diameter which appeared similar to that of a major coronary artery	6
The left main coronary artery exhibited a short course measuring between 3 and 5 mm, associated with a long proximal segment of the left anterior descending artery	5
The anterior descending and circumflex arteries originated from separate aortic ostia	3

only 36.2% blood to the myocardium.[7] Of note is the observation that the left main coronary artery carries approximately five times the amount of blood flow to the left ventricle compared to the right coronary artery, being the main source of oxygen and nutrients to the walls of the left ventricle and interventricular septum. Occlusion of the right coronary artery which takes no part in the blood supply of the left ventricle may cause no detectable myocardial necrosis. On the other hand, when the angiographic catheter is introduced into the aortic ostium and produces complete occlusion of the left main coronary artery, the clinical consequences are potentially catastrophic; the patient may experience dramatic falls in both flow and pressure and even fatal cardiac arrest.

The diagnosis of stenotic lesions of the left main coronary artery ultimately depends on coronary angiography and/or is confirmed by post-mortem gross inspection. The prognosis of these stenoses is poor and the mortality is high, particularly in patients with left anterior descending and left circumflex arteries unsuitable for surgery.[8] In the general population of patients undergoing coronary angiography, the prevalence of stenosis of the left main coronary artery ranges from 2 to 10%.[9-11] Complete occlusion of the left main coronary artery was identified in two male subjects of 2.546 patients undergoing cardiac catheterization over a period of 14.5 years.[12] This was associated with the significant development of collateral vessels from the right coronary arterial system, as documented by cineangiography. The presence of these collateral vessels is considered to be of particular significance in the survival of patients.

The left main coronary artery has its origin in a rapidly tapering, well-shaped ostium, situated near the downstream end of the left sinus of Valsalva. Its long transverse axis is perpendicular to the long axis of the ascending aorta,[13] but important variations have been recorded, a mean of 46° being detected in some resin casts.[14] In 20% of cases this angle was found to be more than 80°.[13] The elliptical orifice of the left main coronary artery is usually larger than the orifice of the right coronary artery, being 4.7 mm in width and 3.2 mm in height, compared to 3.7 and 2.4 mm of the right coronary artery.[2]

Several types of minor anomalies may be detected by the pathologist during routine autopsy as regards the number of ostia (independent origin of the left anterior descending and/or left circumflex arteries), and as regards the position of ostia (high take-off of the left main coronary artery with the orifice showing a funnel-like dilatation). The presence of an elevated orifice may be associated with a coronary impairment of blood flow because of unfavorable mechanical factors resulting in turbulence and skimming of blood. An ectopic ostium, associated with atresia of the left coronary ostium and with occlusive intimal thickening was presented. Both myocardial infarction and sudden cardiac death in association with hypoplastic left main coronary artery have been reported.[15]

The atherosclerotic plaques prevalently develop in two zones of the aortic origin of the left main coronary artery: the superior ostial zone of the outer wall and the postostial zone of the inner wall.[13] It has been suggested that the proximal ostial margin is more exposed to the systolic thrust than the inferior ostial margin. This seems to be due to the protective position of the aortic cusp which covers the inferior ostial margin during the systolic phase.

At autopsy the ostium of the left main coronary artery can be gently probed for evidence of ostial stenosis produced by atherosclerotic plaques or anatomical anomalies, using metal catheters of various calibers. The length of the vessel is not difficult to estimate in each case, nor is the angle between the left anterior descending and left circumflex arteries. In our material this angle ranges from 45 to 95° with a mean value of approximately 70°. A distinctive distribution pattern of atherosclerotic lesions was detected in the bifurcation area of the left main coronary artery which may be influenced by the angle of bifurcation. The lesions develop with a high frequency on the outer walls of the bifurcation and at the inner curvature downstream of the bifurcation. On the other hand, the lesions were uncommon on the inner walls distal to the bifurcation.[16]

An anomaly which must be identified during routine autopsy is the origin of the left main coronary artery from: (1) the right sinus of Valsalva; (2) the right coronary artery: or (3) the pulmonary artery. Of particular importance for the pathologist to explain the cause of death of a young or very young subject is a main coronary artery arising from the pulmonary artery, since this type of anomaly may give rise to myocardial infarction in the first year of life. Those patients who survive to adulthood remain at very high risk for myocardial infarction and sudden cardiac death. In these patients blood is supplied to the perfusion area of the left main coronary artery through collateral vessels from the right coronary artery. Therefore, the blood is insufficient in the major part of the left ventricular myocardium being further diminished by the occurrence of an arteriovenous shunt of the pulmonary artery leading to coronary steal. This is why cases with anomalous origin of the left main coronary artery from the pulmonary artery seldom reach adulthood. They may exhibit cardiomegaly, subvalvulvar mitral insufficiency, left ventricular failure, clinical symptoms, and electrocardiographic signs of myocardial infarction and/or life threatening arrhythmias.

With the increasing use of coronary angiography the significance of many anomalies and even of some as yet overlooked anatomic peculiarities are becoming better appreciated from a clinical point of view. Thus, it has been demonstrated that in some cases the left main coronary artery, after arising anomalously from the right sinus of Valsalva, passes between the pulmonary artery and the aorta. Exertion-related sudden death in such cases has been linked to the retropulmonary arterial course of the left main coronary artery, since no instances of premature sudden death have been recognized when the left main coronary artery courses anterior to the pulmonary artery. This led to the conclusion that only in the retropulmonary arterial course might the vessel have been subjected to squeezing with resulting myocardial clinical manifestations. It is tempting to think and speculate that the sulcus between the aorta and pulmonary artery produces a particular syphon-like kink. Secondary to this suggested constriction of the left main coronary artery near its origin, anginal pain, ventricular fibrillation, anterolateral, or anteroseptal myocardial infarction may possibly occur. The

potentially lethal consequences of the retropulmonary arterial course of the left main coronary artery have been attributed to compression of the left main coronary artery secondary to simultaneous exaggerated distension of both aorta and pulmonary artery during vigorous exercise and to an acute angulation of the left main coronary artery at its anomalous site of origin. Viewed from within the aorta this acute angulation imparted a slit-like appearance to the ostium. The existence of this ostial valve-like region associated with acute angulation may predispose to both myocardial infarction and sudden cardiac death. The possibility that an increased ventricular activity may also favor the flap-like closure of the coronary aortic ostium cannot be neglected. Irrespective of the main mechanism involved, this closure seems to be a reversible phenomenon, as demonstrated by many patients with this minor anomaly who have experienced exertion, chest pain, and arrhythmias before death.

In essence, the pathologist must record if dislocation of the ostium exists and particularly if the left main coronary artery arises from an aortic sinus other than the usual. It is also important to note if the respective ostium is located above or below the usual level to such an extent that it may be hidden by the valve leaflet during ventricular systole. A second important observation refers to the initial course of the left main coronary artery, which may encircle the pulmonary artery anteriorly or posteriorly.

In 1/600 to 1/3000 autopsied cases the existence of a single coronary artery has been reported. This anomaly is compatible with an adequate myocardial blood supply, but the occurrence of stenotic or occlusive atherosclerotic plaques in this unique vessel may be compared to bilateral obstruction of both left main trunk and right coronary artery. When a single coronary artery exists and arises from the left aortic sinus, it usually assumes a left pattern of distribution similar to that of the left main coronary artery. In such cases the right coronary artery can be seen as a branch of the left anterior descending artery. When a single coronary artery exists and it arises from the right aortic sinus, it encircles the heart, courses in the atmoventricular groove, and ends as the left anterior descending artery.

A study in our laboratory showed that the left main coronary artery with its usual origin, course, and length, has a particular and unexpected resistance to both intimal thickening and atherosclerotic involvement, as compared to the left anterior descending artery.[17] The first atherosclerotic plaques develop about 3 decades later in the left main coronary artery then in the proximal segment of the left anterior descending artery (Table 3). In the 51 to 55 year old age group, only 13.3% of subjects showed atherosclerotic plaques in the left main coronary artery, compared to 77.6% in the proximal segment of the left anterior descending artery. This natural resistance to intimal thickening and plaque development revealed in post-mortem studies is in agreement with the rarity of stenotic lesions detected angiographically. This particular resistance may be related, at least in part, to the fact that the left main coronary artery is a transitional segment between the aorta, a pure elastic vessel, and the left anterior descending artery, a pure muscular vessel. In this transitional segment, particularly in its upper half, the number of elastic membranes of the media diminishes, alternating layers of elastic membranes and smooth muscle cells being present, with a net prevalence in certain areas of longitudinal muscle columns (Figure 32).

A left main coronary artery less than 5 mm in length was associated in our material with an accelerated atherosclerotic involvement in the proximal segment of both left anterior descending and left circumflex arteries.[4] This minor anomaly was detected in 5% of unselected cases or in 1 out of every 20 subjects (Figure 33). In other studies this deviation from the normal length was seen in one out of every five individuals.[18] When the left main coronary artery is short, a long proximal segment of the left anterior descending artery is present and can be subjected, through systolic kinking, to unusual hemodynamic stresses. This part of the vessel, tethered proximally only by the left main coronary artery and anchored distally to the myocardium by the first septal vessel, may show abnormal bending and kinking which may enhance atherosclerotic involvement. The attention of certain investi-

Table 3
PERCENT OF SUBJECTS WITH INTIMAL THICKENING (i.th.) AND ATHEROSCLEROTIC PLAQUES (a.p.)[a]

Age groups (years)	Left main coronary artery at 5 mm		Left anterior descending artery at 20 mm	
	i.th.	a.p.	i.th.	a.p.
11—15	0	0	94	0
16—20	0	0	100	10
21—25	17	0	100	23
26—30	27	0	100	25
31—35	30	6	100	46
36—40	45	5	100	53
41—45	65	7	100	64
45—50	70	13	100	70
51—55	92	18	100	78
56—60	98	27	100	82

[a] At 5 mm distal to the aortic point of origin of the left main coronary artery and at 20 mm distal to the point of origin of the left anterior descending artery from the left main coronary artery.

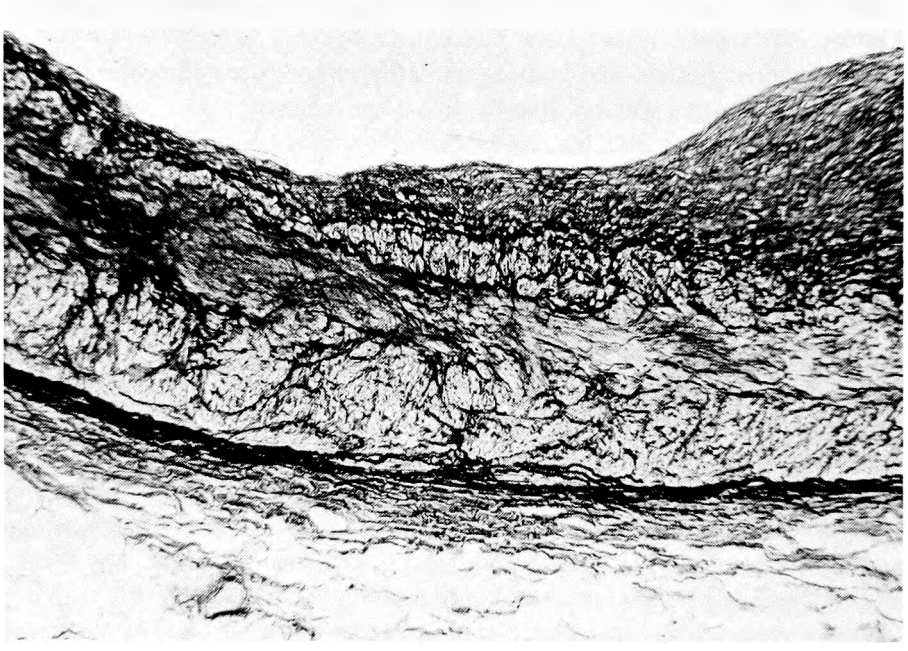


FIGURE 32. Left main coronary artery in an apparently healthy female subject 52 years old who died in a car accident. Prevalence of longitudinal muscle columns in the basal zone of the media and at the media-intima frontier. Passage from an area without intimal thickening (left) to an area with a fibromuscular plaque (right). (Resorcin fuchsin-alcian blue. Magnification × 220.)

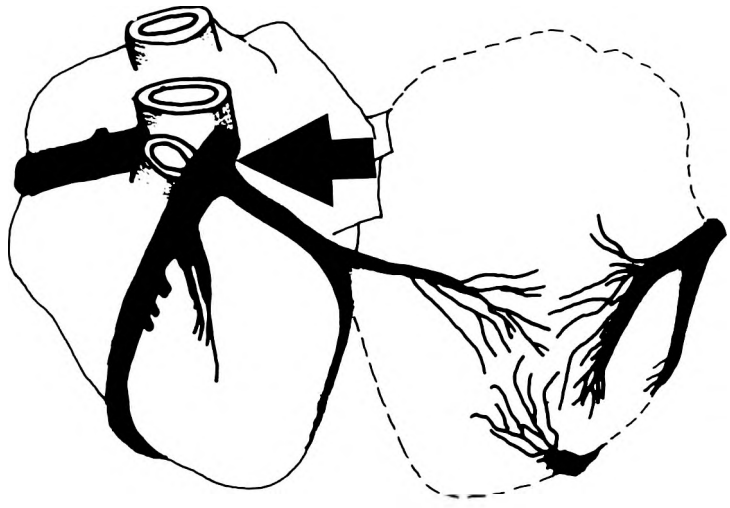


FIGURE 33. In 5% of our unselected cases, an atherogenic deviation from the common type of distribution of the coronary arteries was seen, characterized by a short (less than 5 mm) left main coronary artery (arrow). (From Velican, D. and Velican, C., *Atherosclerosis*, 40, 309, 1981. With permission.)

gators has been focused on the atherosclerotic plaques which develop at the branch site of the first septal vessel, because if they encroach upon the vessel lumen, they may impair the blood supply of the bundle of His and other structures of the conduction system located within the interventricular septum.[19] This may also be related to the observation that a short left main coronary artery was present in a great proportion of patients with complete left-bundle branch block.[20] The length of the left main coronary artery has also been correlated in certain studies with the pattern of coronary arterial dominance. Based on its origin, the prevalent type of the coronary circulation was divided into right preponderant, left preponderant, and balanced circulation.[21-23] The artery that gives rise to the posterior descending vessel was usually designated the dominant artery. Only 10 to 15% of hearts show left coronary arterial dominance and this was correlated with a left main coronary artery which was significantly shorter than that of individuals with a dominant right coronary arterial circulation or with a balanced circulation.[24] The attempts to relate the length of the left main coronary artery and heart weight gave negative results; on the other hand, the left main coronary artery appeared slightly longer when the heart was enlarged.[25]

In essence, it is tempting to presume that the longer the left main coronary artery, the shorter the proximal segment of the left anterior descending artery and vice versa. A long left main coronary artery may be expected to "protect" the bifurcation of the vessel and the proximal part of both left anterior descending and left circumflex arteries from the atherogenic effects of hemodynamic stresses by reducing pressure and flow rate and by damping pulsatile pressure and flow. If this view is accepted, the length of the left main coronary artery should be regarded as an inherited characteristic influencing the rate of development and severity of atherosclerotic plaques; likewise, the geometry of the left main coronary artery bifurcation should be regarded as an inherited characteristic which may strongly influence the hydraulic conditions and the atherogenic effects of certain hemodynamic stresses. This is supported by the results of studies which demonstrated that the distribution of lesions around the bifurcation is a function of its geometry.[26,27] If the results of mathematical analyses and experimental flow studies are taken into consideration, then the above-mentioned investigations suggest that localization of atherosclerotic lesions coincides with regions of low flow velocities.

The role of genetic factors in atherogenesis is also supported by the observations which revealed that when the angle of bifurcation increases and becomes less acute, the lateral wall of both left anterior descending artery and left circumflex artery seem to be more prone to atherosclerotic involvement.[16]

Before closing this presentation on the anatomy of the left main coronary artery as related to atherosclerotic involvement and the natural history of coronary atherosclerosis, it is of interest to remember the attempts made to visualize noninvasively the left main coronary artery in the living individual using a cross-sectional echocardiographic technique. The artery appears as two dominant parallel linear echoes, separated by a clear space representing the lumen of the vessel. Apical echocardiographic techniques and exercise ^{201}Tl myocardial imaging were also used for identifying obstructions of the left main coronary artery.[28,29]

3. Left Anterior Descending Artery

This vessel seems to continue the left main coronary artery and is considered the major coronary artery with the most constant origin and course, with an external diameter which ranges from 2 to 5 mm.

The left anterior descending artery gives rise to several important branches, reducing its own diameter significantly and continuously over its entire length. This contrasts with the less significant changes in geometry and size of the right coronary artery which may reach the crux cordis appearing as a large vessel. Whereas the areas supplied by the right coronary artery and the left circumflex artery vary to a great extent, the areas supplied by the left anterior descending artery are almost constant: the apex, the anterior and lateral wall of the left ventricle, about one third of the anterior part of the right ventricle, and about two thirds of the ventricular septum. In fact, the anterior part of the interventricular septum and the anterior wall of the left ventricle constitute about 40% of the total left ventricular myocardium.[30] Consequently, the presence of stenotic plaques in the left anterior descending artery is a high-risk change and the patients with unstable angina and such lesions are candidates for reperfusion.[31]

The region from the bifurcation of the left main coronary artery into the left anterior descending artery and left circumflex artery to the take-off of the first septal branch is usually designated the proximal segment, the area from the first septal branch to the take-off of the second diagonal is usually designated as the middle segment and the remaining artery can be considered the distal segment.[32]

A long left anterior descending artery is frequently associated with a short posterior descending artery (occurring as a branch of the right coronary artery) and vice versa. In our material a very long and large left anterior descending artery coexisting with a short posterior descending artery was detected in 7% of unselected subjects (Figure 34).[4] Also in unselected cases the left main coronary artery trifurcated in 6.5% of subjects (Figure 35). In addition, in 24% of cases we observed a second vessel coursing adjacent to the anterior interventricular groove, running in a caudal direction toward the apex. Its presence was also mentioned by other investigators.[33-36] The most frequently visualized aspect is an early bifurcation of the proximal segment of the left anterior descending artery, one vessel remaining in the anterior interventricular sulcus, and the other showing a variable course outside the sulcus. This peculiar branching pattern is also called the "dual anterior descending artery" and its recognition is necessary to prevent errors of interpretation and unrealistic anatomo-clinical correlations. In the presence of one left anterior descending artery, a stenotic lesion may represent a very high risk lesion; in the presence of two left anterior descending arteries, an occlusive lesion in one of the two vessels running toward the apex may be without pathophysiological significance if the second artery plays the role of a bypass vessel. In such conditions, the myocardium may appear unaltered despite the occlusion visualized by the pathologist. The existence of this variant is also important for angiographers to prevent

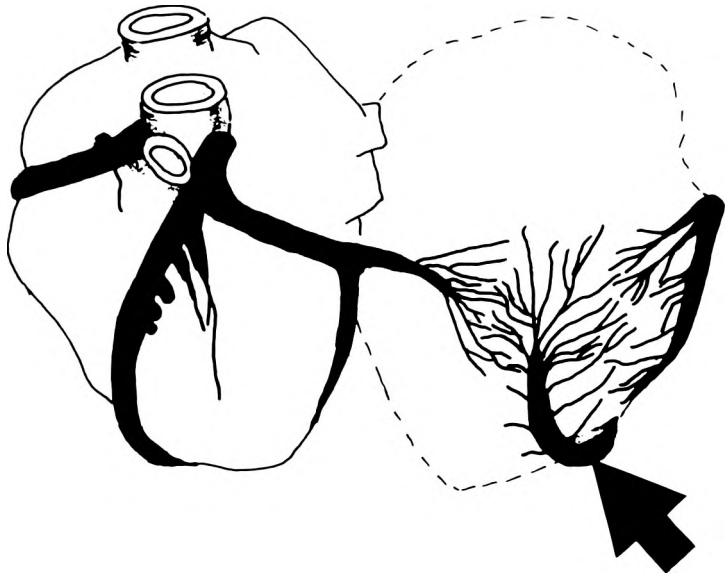


FIGURE 34. In 7% of our unselected cases a very long left anterior descending artery was visualized which coursed around the apex (arrow) and ascended in the posterior interventricular groove to the area of the crux cordis, giving rise to terminal branches in a region usually supplied by the posterior descending branch of the right coronary artery. This minor but atherogenic deviation was associated with the presence of one or two thin and short posterior descending branches.

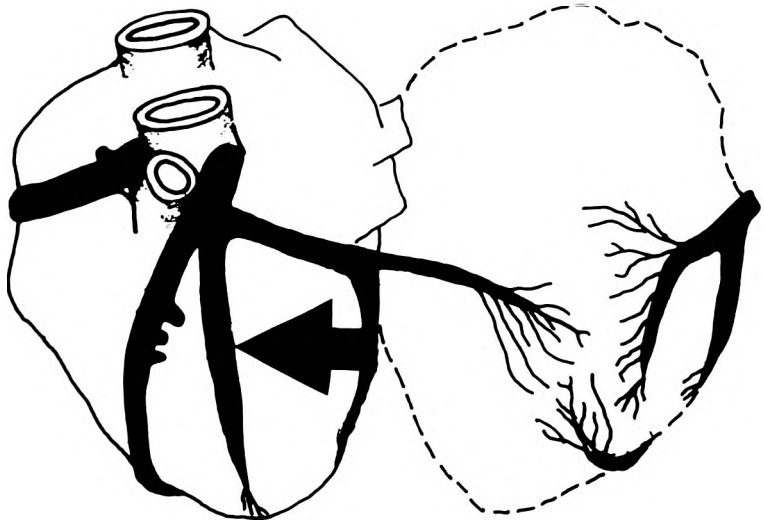


FIGURE 35. In 6.5% of our cases the left main coronary artery trifurcated and gave rise to large diagonal vessel (arrow) susceptible to a rapid atherosclerotic involvement.

errors of interpretation of the coronary arteriogram and for planning optimal surgical therapy. Whenever discrepancies exist between the severity of left anterior descending artery lesions and the absence of changes in regional left ventricular wall motion, the possible presence of a dual left anterior descending artery must be carefully investigated.

It is generally agreed that the left anterior descending artery is the vessel of the coronary arterial bed which exhibits the greatest susceptibility to atherosclerotic involvement and

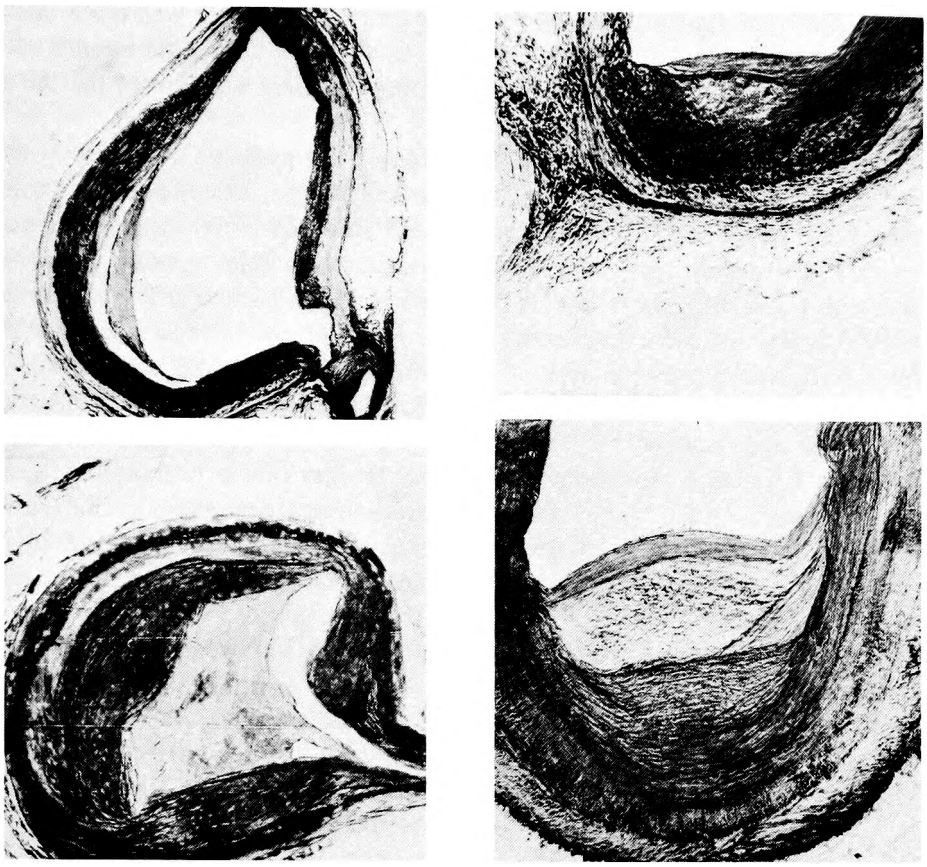
particularly to the onset of atherosclerotic plaques of possible clinical significance. Moreover, certain studies revealed that an angiographically significant narrowing involving the proximal segment of the left anterior descending artery is highly correlated with an increased 1 to 3 year mortality rate.[37]

Our studies demonstrated an unexpected precocity, intensity, and severity of the early age-dependent changes in the left anterior descending artery during pre- and postnatal periods.[38] Likewise, our studies showed in 7% of cases the existence of a very large and long left anterior descending artery which coursed around the apex and ascended to the area of the crux cordis usually supplied by the posterior descending branch of the right coronary artery. On the other hand, one or two short and narrow posterior descending branches coexisted with this large and long left anterior descending artery (Figure 34). Comparison with the corresponding control subjects revealed a more rapid, extensive, and severe intimal thickening in both proximal and intermediate segments of the left anterior descending artery, as well as in the proximal segments of the first diagonal and first septal vessels. A more rapid onset of atherosclerotic plaques was also found in the above-mentioned vascular segments. There was, likewise, an obvious difference in the rate at which the obstructive character of atherosclerotic plaques increased in successive age groups.[4] Comparison of cases of similar age, sex, cause of death, family history, and environmental conditions with and without the above-mentioned atherogenic deviation involving the left anterior descending artery suggests that some genetically transmitted changes in the length, size, and type of branching might lead to an accelerated atherosclerotic involvement and might act as an endogenous risk factor for coronary heart disease. One may speculate that such atherogenic deviations could explain some familiar clustering in coronary heart disease.

An intriguing result was furnished by cineangiographic studies which showed that the left anterior descending artery undergoes a "paradox range of motion" with respect to the other major coronary arteries.[39] During systole the major coronary arteries are tortuous, but not the left anterior descending artery; on the other hand, during diastole the major coronary arteries are generally straight, while the left anterior descending artery is tortuous. This was related to the off-axis forces exerted on this vessel by its main branches during cardiac cycle and was associated with particular hemodynamic stresses acting as atherogenic agents.

The presence of particular hemodynamic stresses acting as atherogenic agents could be also related to the existence of many important branch sites. These branch sites may produce the breakup of the flow pattern, for it is just beyond the branches that disturbed flow commences.[40,41] At the level of the branch site of the first septal vessel special conditions of flow seem to exist which favor incorporation of microthrombi and occurrence of intramural thrombi (Figure 36).[42] This narrowing of the proximal segment of the left anterior descending artery just before or at the level of the branch site of the first septal vessel is considered by certain investigators as an independent predictor of mortality.[43]

It is interesting to note that using special methods, a clustering of lesions of possible clinical significance was demonstrated to appear in the same susceptible area between the branch sites of the first diagonal and first septal vessels and at the level of the respective branching areas.[44] In the International Atherosclerosis Project Study, a high prevalence of so-called raised lesions was found from the first to the second centimeter of the proximal segment of the left anterior descending artery.[45] This area also included the branch sites of the first diagonal and septal vessels. Not only was the incidence of thrombotic events recorded to be much higher than in other major coronary arteries in the above-mentioned region of the left anterior descending artery, but also the association of these thrombotic events with fatal myocardial infarction.[46] Moreover, an angiographic study on resuscitated patients from ventricular fibrillation showed that 14% had isolated left anterior descending artery narrowing.[47] The survival of patients with severe ventricular arrhythmias seemed to be inversely correlated with the degree of narrowing of the proximal segment of the left anterior de-



A B

FIGURE 36. (Top) The anterior descending artery at the branching site of the first septal vessel in a male subject 28 years old (A) and in a male subject 37 years old (B). (Bottom) A similar topographic site in a male subject 49 years old (A) and 50 years old (B). Suggested aspects of successive microthrombi encrustation and organization leading to the occurrence of fibrohyaline plaques, usually lipid-free. (From Velican, C. and Velican, D., *Atherosclerosis*, 54, 333, 1985. With permission.)

scending artery.[48] All these results might be associated with that fact that a stenotic atherosclerotic plaque of the left anterior descending artery, which is not by-passed by collateral vessels, may put at risk more than 30% of the myocardium of the left ventricle.[49]

A peculiar observation is that women using oral contraceptives have a preponderance of isolated left anterior descending artery stenoses or occlusions.[50]

The anatomical branching pattern of the coronary arteries also include vessels which pass through muscular tunnels, and these so-called "myocardial bridges" might be considered an additional genetic factor which may influence the susceptibility to atherosclerotic involvement. The term myocardial bridge was mainly used during the last two decades in an angiographic sense to describe an angiographic entity characterized by systolic narrowing of the left anterior descending artery.[51] The role of such cyclic narrowings in the pathogenesis of angina pectoris and other myocardial clinical manifestations was largely suggested. In an anatomical sense the term myocardial bridge is sometimes used similarly to intramural coronary artery, mural coronary artery, coronary artery overbridging, myocardial loop, etc. In some animals (hamster, squirrel, rabbit, rat, guinea pig), the coronary arteries are entirely intramyocardial; in others (pig, cow, horse), entirely epicardial, whereas in man they are predominantly epicardial but exhibit frequent myocardial bridging.[52]

The intramyocardial course of the left anterior descending artery was more analytically investigated by Crainicianu, in 1922.[53] It became a subject of radiologic interest starting in 1960 when a "transient" functional occlusion in the proximal segment of the left anterior descending artery during systole was reported.[54]

When examining the left anterior descending artery the pathologist must pay particular attention to the possible presence of these myocardial bridges, since they may be involved in myocardial infarction and sudden cardiac death.[55] Reduced blood supply, particularly to the interventricular septum, may be caused by segmental tubular systolic compression of the left anterior descending artery and its first septal branch, particularly when there is also a diminutive or absent posterior descending artery. The intramural course entails an abnormal angulation which, associated with systolic compression, may produce myocardial ischemia manifested as symptoms of angina pectoris which disappear following myotomy of the overbridge in selected patients.[56,57]

Autopsy data revealed a frequency of muscular bridges of the left anterior descending artery ranging from 5.4 to 85.7%.[58,59] This wide variation clearly depends upon the awareness and prosecting technique of each pathologist. Routine gross inspection may reveal myocardial bridges in only 6% of cases, whereas a meticulous anatomical dissection may disclose the bridges in 60% of cases.[52]

Myocardial bridging of the left anterior descending artery has been classified as proximal, middle, and distal. When myocardial infarction occurred in cases having bridges, it was almost confined to those in the proximal group; in addition, hypertension may enhance infarction in the case of myocardial bridges in the proximal segment of the left anterior descending artery.[60]

Recent findings support the observation made more than 3 decades ago that during its intramural course the left anterior descending artery usually exhibits a thin wall, often without an intimal layer, and an unexpected resistance to atherosclerotic involvement even in advanced age groups.[61] Myocardial bridges seem able to "protect" the respective segment of the left anterior descending artery from development of obstructive atherosclerotic lesions. This has also been commonly seen by surgeons who performed coronary bypass in patients with an intramural segment of the left anterior descending artery. It appears likely that myocardial bridges could also protect the distal segment of the left anterior descending artery, but increases the susceptibility to atherosclerotic involvement in the premural portion of the major coronary artery, just before penetration into the muscular tunnel. This difference in the susceptibility to the onset of atherosclerotic plaques between premural and intramural segments of the same left anterior descending artery is a fascinating subject of research related to the natural history of coronary atherosclerosis and also to its prevention.

4. Diagonal and Septal Branches

They originate from the proximal and intermediate segments of the left anterior descending artery and have a diameter of 0.5 to 3.0 mm. The diagonal branches reach the left ventricular wall obliquely and in a study on patients who died of coronary heart disease, ramus diagonalis from the left anterior descending artery was found to be involved by atherosclerotic lesions to the same extent as the parent vessel.[62] In our material this branch was mainly involved by stenotic atherosclerotic plaques and by occlusive lesions in patients with transmural myocardial infarction of the left ventricle, especially in cases with lateral and anterolateral infarction. A suggestion has been made that the size of these infarcts demonstrate that the perfusion area of the first diagonal branch may be as large as that of the distal segment of the left anterior descending artery.[63] In a recent study, our laboratory showed that in an unselected Bucharest population sample of patients who died from accidental causes (mainly traffic accidents), atherosclerotic plaques could be detected in the first diagonal branch in males aged 21 to 25 years and in females aged 26 to 30 years. Intimal thickening was found

in the first diagonal branch in 8% of cases in the 11 to 15 year old age group, 19% in the 21 to 25 year old age group, 38% in the 31 to 35 year old age group, and 49% in the 41 to 45 year old age group.[64,65] This would indicate that approximately half of apparently healthy middle-aged adults have intimal thickening of the first diagonal branch. In the same study we showed that the intima/media thickness ratio surpassed unity in subjects aged 31 to 35 years and rose to 2.58 in the 46 to 50 year old age group. This intima which is 2.5 times thicker than the underlying media produced a more than 50% reduction in the luminal diameter.

Investigations carried out on other population groups revealed that in 24% of cases the first diagonal branch had a diameter of more than 2.0 mm, like a major coronary artery, and in only 9% of cases was it hypoplastic.[66] We also observed that in one out of every four cases the first diagonal branch has a diameter similar to a major coronary artery and we are unable to understand why this vessel is systematically neglected in many studies on the atherosclerotic involvement of the coronary arteries. The diagonal branches range in number from one to six, but for the pathologist, the first and sometimes the second vessel may have particular importance in atherosclerotic involvement and myocardial clinical manifestations.

In 10 to 15% of individuals the first diagonal branch may arise during the intramural course of the left anterior descending artery; in 6.5% of cases we revealed that the left main coronary artery trifurcated, the highest located diagonal branch occurring as an artery comprised in the angle subtended by the left anterior descending and left circumflex arteries (see Figure 35). When the left main coronary artery trifurcated, the first diagonal branch showed an external diameter from 2 to 3 mm and usually descended vertically and not diagonally across the lateral wall of the left ventricle, appearing as a duplicate of the left anterior descending artery.

In essence gross inspection of the proximal segment of the first diagonal artery has to be a routine examination and is absolutely necessary in all patients with lateral and anterior infarctions. In cases with more than 75% narrowing by atherosclerotic plaques of the first diagonal branch, a meticulous investigation may identify small areas of myocardial necrosis and fibrosis of the lateral and/or anterior wall of the left ventricle. In a study on the "neglected coronary atherosclerosis" we recorded atherosclerotic plaques in the first diagonal branch in 8% of apparently healthy subjects aged 46 to 50 years who died from accidental causes, and in 16% of patients who died from myocardial infarction (Figure 37).[67]

The main septal branch, usually the first or second septal vessel, has a diameter of 0.5 to 3 mm, arising in the proximal 2 cm of the left anterior descending artery in the anterior interventricular groove, before the intramural course or sometimes during its intramural course.

In approximately 30% of patients undergoing coronary angiography for evaluation of myocardial clinical manifestations, the first septal artery exhibits a luminal diameter of 1.5 mm or more.[68] In a post-mortem study a main septal branch of 1.5 mm or larger was found in 64% of dissected human hearts.[2] Since the canine experimental model is frequently used in the study of coronary heart disease and particularly of myocardial ischemia, it may be of interest to remember that the canine septal artery is usually a well-developed vessel. Approximately 16% of left main coronary artery flow enters this septal vessel which perfuses 70 to 75% of the interventricular septum. It also contributes approximately 25% of the collateral circulation to the other major coronary arteries.[69] Total native collateral flow through the septal artery is one half that through the circumflex artery and two thirds that of the left anterior descending canine coronary artery. Temporary occlusion of the septal artery in experimental models may produce nontransmural septal infarction.[70]

The first septal vessel anchors to the myocardial surface the proximal segment of the left anterior descending artery which is submitted to torsion and greater bending and kinking

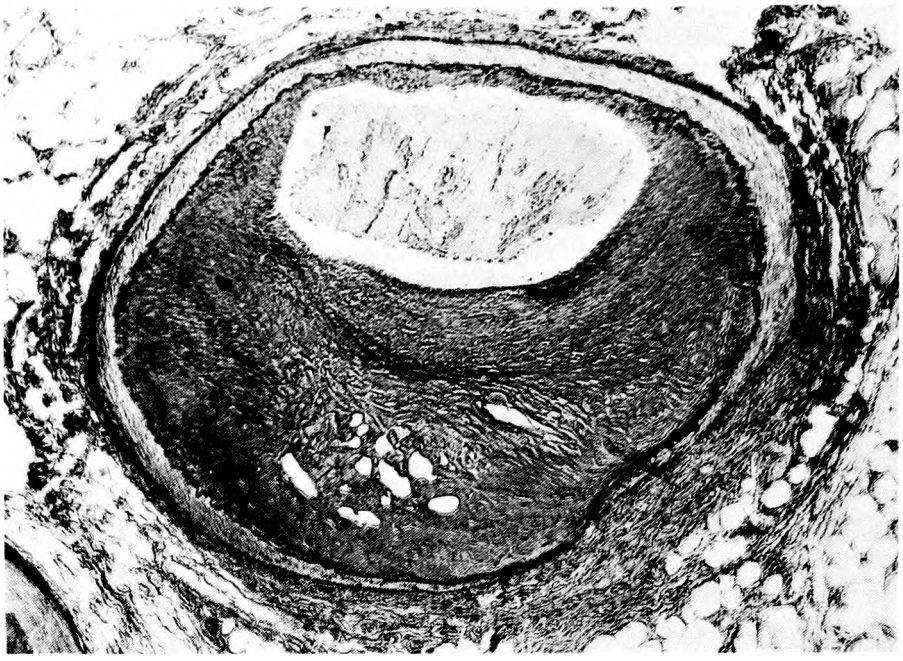


FIGURE 37. A grossly neglected occlusive plaque of the first diagonal artery in a 57-year-old male patient who died of an extensive myocardial infarction of the anterior wall of the left ventricle. Routine gross inspection did not reveal obstructive lesions in the major coronary arteries. Without our additional light microscopic examination, the occlusive plaque would remain a neglected lesion and the respective case would be classified as infarction without occlusive lesions in the coronary arteries. (Resorcin fuchsin-alcian blue. Magnification × 120.)

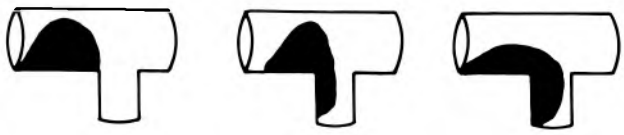
during each myocardial contraction. Maximum hemodynamic and mechanical stresses are considered to fall upon the proximal segment of the left anterior descending artery just where it becomes attached to the myocardial surface by the first septal vessel.[71] At this level, stenotic atherosclerotic plaques frequently develop and we have also found at this site an unexpected frequency of intramural thrombi (see Figure 36).[64,65]

Intimal thickening was detected in our material in the first septal branch as early as the age of 6 to 10 years, the percentage of subjects with such intimal thickening ranging from 5% in the 11 to 15 year old age group to 33% in the 46 to 50 year old age group. The intima thickness vs. media thickness ratio also augmented from 1.78 in subjects aged 11 to 15 years to 2.56 in subjects aged 46 to 50 years.[42,65]

The first atherosclerotic plaques were recorded in young adults 26 to 30 years old; 12% of mature adults 46 to 50 years old who died in accidents presented atherosclerotic plaques in the main septal branch.[67]

An initial maneuver to reveal the proximal segment of the first septal branch during post-mortem examination is to dissect the proximal segment (2 to 3 cm) of the left anterior descending artery. The easiest septal vessel to deal with is the branch which nearly parallels the parent vessel at a slightly deeper level. Most difficult to identify, to open longitudinally, and to remove for light microscopic examination is the main septal branch which plunges rapidly into the septum at a right angle to the left anterior descending artery. This septal vessel, which arises at about a 90° angle, runs caudal along the septum from front to back, distributing blood to about two thirds of the upper portion of the septum and perfusing almost the entire inferior third.

The main septal vessel mainly supplies the second and third divisions of the right bundle branch and may also provide branches to the first division and even to the bundle of His. The other septal branches are also involved in the blood supply to the anterior and often the



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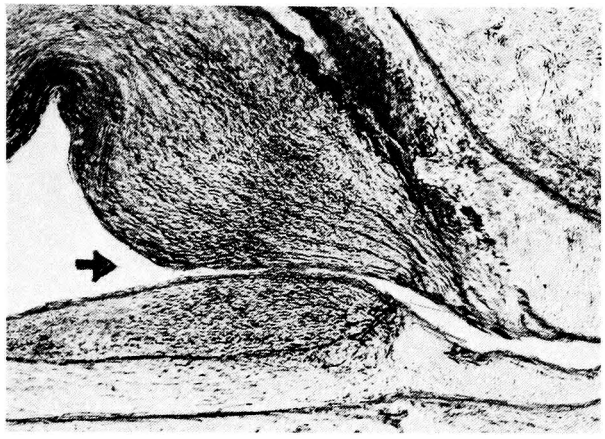


B

FIGURE 38. (A) Schematic presentation of a plaque developed at branch site of the first septal vessel. The respective plaque may alter only the radius of the left anterior descending artery (left); the radii of the parent vessel and first septal branch (middle); it may also encroach upon the lumen of the intercalated vascular segment of the first septal branch (right). (B) Micrograph of the intercalated vascular segment of the first septal branch severely obstructed by an atherosclerotic plaque developed in the left anterior descending artery at the level of the branch mouth (arrow). (Resorcin fuchsin-alcian blue. Magnification × 120.)

inferior papillary muscle. When the posterior descending artery originates from the left circumflex artery, the left main coronary artery becomes the unique source of blood to the entire interventricular septum; a similar situation exists when the posterior descending artery is hypoplastic or emerges as a secondary division of the right marginal artery.

Patchy, irregular areas of healing necrosis were seen in our material in the anterior interventricular septum and left ventricular wall, immediately underneath the branch mouth of the first septal vessel, when a long and narrowed intercalated vascular segment was present.[64] This narrowed intercalated vascular segment of the first septal branch was associated with a very thick intima in the proximal segment of the left anterior descending artery and with the presence of fibronecrotic plaques at the branch site. Such plaque may alter the radius of the parent vessel, the radii of the parent vessel and first septal branch, or particularly the intercalated vascular segment of the first septal branch (Figure 38).

In spite of the frequent presence of myocardial infarctions in the interventricular septum and in spite of the very frequent existence of various types of conduction disturbances, pathologists usually overlook the existence of the main septal branch. It remains unopened during routine gross inspection and is not removed for light microscopic examination, since this is not recommended in the "classical" protocols. The neglected main septal vessel may represent an important weak point for each clinico-pathologic correlation, as suggested by Figure 39.

FIGURE 39. A grossly neglected obstructive atherosclerotic plaque blocking about 90% of the lumen of the first septal artery and extending more than 3 mm along the vessel course is shown of a 61-year-old woman who died of acute anteroseptal myocardial infarction associated with severe arrhythmia. Lumen compromising lesions were absent from the major coronary arteries. (Resorcin fuchsin-alcian blue. Magnification × 80.)

5. Left Circumflex Artery

The vessel diameter varies from 1 to 4.5 mm, the largest size being that of the proximal segment which extends from the bifurcation of the left main coronary artery to the take-off of the left (obtuse) marginal branch. The middle segment is considered from the left marginal to the posterior descending branch and the distal segment the remaining posterior continuation of the left circumflex artery.

The left circumflex artery furnishes 80% of the blood required by the posterior wall and approximately 10% of the blood required by the lateral wall of the left ventricle.[2] Its blood supply to the total left ventricle is considered to represent about one half of that supplied by the left anterior descending artery.

The left circumflex artery must be investigated in the autopsy room from its origin until it is too small to dissect, since many stenotic lesions can be present in the intermediate or even distal segments. Both these intermediate and distal segments are often difficult to find, being buried deep in fat in the posterior atrioventricular sulcus.

The left circumflex artery usually departs at a fairly sharp angle from the bifurcation area of the left main coronary artery to run posteriorly along the atrioventricular groove, toward the crux cordis which it reaches in only 10 to 15% of cases.

In 2 to 6% of individuals, the circumflex artery may arise as a branch of the right coronary artery, whereas the left anterior descending artery seems to originate directly from the aorta and the left main coronary artery cannot be identified (Figure 40). Compared to control subjects, this double deviation was associated, in our material, with a more precocious atherosclerotic involvement in both left anterior descending and right coronary artery, and a slower and less significant development of atherosclerotic plaques in the circumflex artery.[4]

In the greatest proportion of cases, the circumflex artery ends slightly distal to the left margin of the heart, without reaching the posterior interventricular sulcus. Sometimes, before reaching the obtuse (left) margin of the heart, the circumflex artery divides into two parallel

FIGURE 40. In less than 5% of our unselected cases, the circumflex artery arose from the right coronary artery, the left anterior descending artery directly from the aorta, and the left main coronary artery could not be identified.

vessels which occasionally may be of nearly equal size.[71] In certain hearts the circumflex artery terminates as the left (obtuse) marginal branch, supplying only the lateral wall of the left ventricle. This short circumflex artery was revealed in our material in 12% of the cases, its presence being associated with an important development of the right coronary arterial system, particularly of the right coronary artery and its posterior descending branch. In these individuals we detected a more precocious and severe intimal thickening and alterations of the elastic tissue in the proximal and intermediate segments of the right coronary artery and a rapid and extensive development of atherosclerotic plaques. However, in the short circumflex artery, a later and slower development of these plaques was recorded as compared to "control" subjects.[4] On the other hand, in 10% of the cases, the left circumflex artery appeared not only very large, but also with a very long course extending to, or beyond, the posterior interventricular groove, where it gave rise to the posterior descending branch. In such cases the right coronary artery may appear very short, ending as the conus or as the right marginal vessel. In subjects with this type of deviation from the common branching pattern the crux cordis was mainly supplied by the posterior descending branch of the left circumflex artery and also by the terminal segment of the left anterior descending artery. The comparison with the corresponding control subjects revealed:

1. A more precocious and extensive intimal thickening and alterations of the elastic tissue in the proximal and intermediate segments of the circumflex artery
2. Extension of these changes in the paraostial region of the left marginal branch
3. A rapid onset of intimal necrotic areas and fibromuscular plaques in the proximal segment of the left circumflex artery and left marginal branch.
4. Occurrence in mature adults of stenotic atherosclerotic plaques in the intermediate segment of the left circumflex artery, located between the branching sites of the left marginal and posterior descending vessels

The left marginal branch of the left circumflex artery has a diameter of 0.8 to 3.0 mm and in spite of the fact that it may appear as large as a major coronary artery, its presence is usually overlooked by pathologists. It frequently shows an intramural course, being covered by myocardial bridges in a manner similar to the left anterior descending artery. The left

marginal branch reaches the apex in approximately 80% of cases, but only its paraostial region and proximal segment are important in a study on the natural history of coronary atherosclerosis. In apparently healthy subjects who died in accidents we found fibromuscular plaques in the proximal segment of the left marginal branch starting from the age group 21 to 25 years old.[64,65]

In approximately 10% of the cases the left circumflex artery gave rise to the branch of the atrioventricular node; in about 40% of the cases one or several branches supplying the sinus node can be visualized emerging from the left circumflex artery. These branches may exhibit a very thick intima encroaching upon 50 to 75% of the vessel lumen in the absence of visible atherosclerotic plaques.[65] The left circumflex artery may also give rise to branches which represent an important connection between the proximal segment of the circumflex artery and the proximal and intermediate segments of the right coronary artery, such as Kugel's artery.[72]

Very rarely has the pathologist the occasion of seeing a circumflex artery arising from the right sinus of Valsalva; such "anomalies" were revealed angiographically in only 0.6% of cases.[73]

Isolated severe atherosclerotic involvement of the left circumflex artery could not be revealed in our material since in middle-aged subjects, a certain degree of atherosclerotic involvement was present in all major coronary arteries.

Percutaneous transluminal angioplasty was performed in patients considered to have a single vessel disease, occurring angiographically as "isolated" circumflex artery stenosis with more than 70% luminal narrowing. This apparently isolated disease of the left circumflex artery did not appear to cause severe left ventricular dysfunction — only electrocardiographic abnormalities in nearly all patients.[74]

In many instances, posterior myocardial infarction was present only when both left circumflex and right coronary arteries were occluded. In hearts with a short right coronary artery, occlusion of the left circumflex artery appeared to be the major pathogenetic mechanism of posterior myocardial infarction, and vice versa.[75]

In essence, for the routine pathologist it is important to know that *the circumflex artery is the vessel of the coronary arterial tree exhibiting the most variable origin (left main coronary artery, aorta, right coronary artery), extramural course, topography, length, and branching pattern*. It may occur as a very long or a very short vessel, as a large or diminutive vessel, or as a vessel with or without important branches. Therefore, the mean values obtained on the circumflex artery on a group basis, as concerns the intimal surface involved with atherosclerotic lesions, or the degree of luminal narrowing or of intimal thickening, are difficult to evaluate and to compare.

6. Right Coronary Artery

The right coronary artery differs from the left main coronary artery not only in its aortic origin, but also in its innervation, microarchitecture of the medial smooth muscle cells, and thickness of the adventitia. Frequently, a large circumferential muscle mass can be seen at the aortic orifice of the right, but not of the left coronary artery. The right coronary artery and left main coronary artery undergo different ranges of motion during each cardiac cycle, the right coronary artery exhibiting greater angulation, tortuosity, and lateral motion.[39]

Since the right coronary artery gives rise to the branch of the sinus node in more than 60% of cases and to the branch of the atrioventricular node in about 90% of cases, it may be considered the vessel of the coronary arterial tree mainly involved in the blood supply of the conduction system. On the other hand, its blood supply to the left ventricle is sometimes negligible and of minor importance compared to that of the first diagonal, first septal, and even marginal vessels. The right coronary artery carries approximately five times less blood to the left ventricle as compared to the left main coronary artery and when the right coronary arterial system is well developed it can supply only one third of the myocardium.[7]

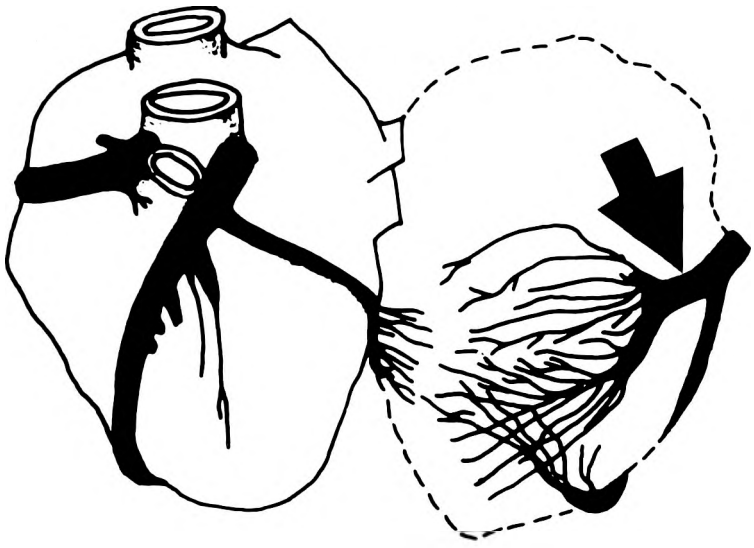


FIGURE 41. In 12% of our unselected cases, the right coronary artery showed a considerable development, reaching the crux cordis as a large vessel (arrow). This minor atherogenic deviation from the common type of distribution of the coronary arteries was associated with a narrow and short left circumflex artery which may end as the left marginal branch.

The right coronary artery is a large vessel (2.5 to 5 mm) which must be carefully investigated from its aortic origin to the area of the posterior interventricular groove. Its proximal segment extends up to the origin of the right (acute) marginal branch, its intermediate segment up to the origin of the posterior descending branch, and the remaining segment is the distal segment. The posterior wall of the left ventricle is in more than two thirds of the cases supplied by this terminal segment of the right coronary artery; in such hearts, the posterolateral branch of the circumflex artery is small or rudimentary.

The right coronary artery is, as a rule, very long and large and can be opened longitudinally up to the crux cordis. Examination of the intermediate segment must be made in each case, since this intermediate segment is the most susceptible to atherosclerotic involvement. In addition, the paraostial region and proximal segment of both right marginal and posterior descending vessels show both intimal thickening and atherosclerotic plaques. On the other hand, our studies revealed that the first centimeter of the right coronary artery usually showed a certain resistance to atherosclerotic involvement.[17] This initial portion exhibits an intermediate microarchitecture between an elastic and a muscular vessel. The above results clearly show that it is not possible to deduce the severity of the atherosclerotic involvement of the whole right coronary artery by looking only at the proximal segment; the intermediate segment must be also carefully investigated grossly as well as the paraostial region of the right marginal and posterior descending vessels.

In 12% of our unselected cases, the right coronary artery exhibited, starting from childhood, a considerable length, reaching the crux cordis as a large vessel (Figure 41). This long and large right coronary artery coexisted with a large and long right marginal and posterior descending branch, but with a narrow and short left circumflex artery. The large and long right coronary artery showed a more precocious, severe, and extensive intimal thickening and elastic tissue alterations, as well as a more rapid onset and progression of atherosclerotic plaques (particularly in the intermediate segment) than the right coronary artery of subjects of similar age, sex, and race with a common type of distribution of the coronary arteries.[4]

Anomalous origin of the right coronary artery from the left sinus of Valsalva must also

be recorded by the pathologist. Although not previously associated with sudden death or fatal myocardial infarction, this anatomic variant can sometimes be implicated in certain forms of myocardial clinical manifestations of ischemic type. Normally, the right coronary sinus is situated below the sinotubular crest of the right sinus of Valsalva, 10 to 16 mm from the posterior and left aortic valve leaflets. When an anomaly of origin exists, the ostium of the right coronary artery may be located just to the left of the aortic valve commissure separating the left from the right sinus of Valsalva. In cases of the anomalous origin of the left main coronary artery from the right sinus of Valsalva, the right coronary artery forms an acute angle toward the right atrioventricular groove and this acute angulation of the initial portion of the vessel may acquire the aspect of a valve-like ridge and is predisposed to myocardial ischemia and even to sudden cardiac death. The necropsy study may disclose a slit-like right coronary ostium which may be involved in various myocardial clinical manifestations.[76,77]

The pathologist can visualize in about 50% of unselected cases multiple orifices located in the right aortic sinus. Frequently, a double orifice exists from which the right coronary artery and the conus vessel emerge. Whereas variations in the number of orifices for the right coronary artery are common, multiple orifices for the left coronary artery are rare. Cases were presented in which anomalous origin of the right coronary artery from the left sinus of Valsalva was the sole pathologic finding at necropsy.[76]

7. Branches of the Right Coronary Artery

The conus artery, or the third coronary artery, or arteria coronaria accessoria dextra, has a diameter ranging from 0.7 to 2.0 mm, with an average 1.15 mm.[66] It can be visualized as the first branch of the right coronary artery in about 50% of cases, whereas in the remaining 50% it arises from the aorta as an independent vessel, together with the right coronary artery. In certain studies, this vessel is presented as a frequent variant of the first anterior ventricular branch of the right coronary artery that becomes independent and originates directly from the aorta. Its vascular area is the superior one third of the right anterior ventricular wall. In our material this vessel showed particular resistance to atherosclerotic involvement. Even in elderly subjects we were unable to detect occlusive or even severe stenotic atherosclerotic plaques.

Pathologists can visualize this first branch of the right coronary artery at approximately 1 cm from the aortic ostium; it proceeds ventrally and to the left, encircling the outflow tract of the right ventricle at about the level of the pulmonary valve, when it is integrated in the anastomotic circle of Vieussens. The term conus refers conventionally to the muscular cardiac segment interposed between the semilunar and atrioventricular valves; embryologically, it corresponds to the middle third of the bulbus cordis and in adults to the outflow tracts of both ventricles. The conus artery has also been called arteria preinfundibularis.[53] It is an important source of collateral blood flow, its role as a vascular bridge between the right and the left coronary arteries often being emphasized.

Since many occlusive plaques develop at the junction between the proximal; and intermediate segments of the right coronary artery, the functional importance of the conus branch as a collateral vessel will be largely influenced by the topography and severity of the lesions. As long as the branch site of the conus vessel remains nonoccluded by the plaques, its function as a collateral vessel remains unaltered. Moreover, when this function is stimulated by local pressure changes, a rich network of anastomotic channels appears in the anterior wall of the atria, atrial septum, and on the posterior surface of the heart. Unfortunately, the presence of these interconnections cannot be visualized on coronary angiography. They are detected only on post-mortem investigations in which injection and corrosion methods are used.

The sinus node artery is an important second branch of the right coronary artery when

the conus vessel arises from the same right coronary artery; when the conus artery arises directly from the aorta, the sinus node artery is the first branch of the right coronary artery detected by the pathologist (at about 3 to 3.5 mm beyond the aortic ostium). It occurs as a vessel with a diameter of more than 1 mm, which can be isolated over a distance of more than 1 cm. The sinus node vessel runs usually in a direction opposite to the conus branch (cranially, dorsally, and to the right) along the anterior wall of the right atrium, below the right auricular appendage, to reach the ostium of the superior vena cava. The artery passes counterclockwise (sometimes also clockwise) around the superior cavo-atrial junction.

The sinus node vessel must be considered both a main atrial branch, supplying both atria and the atrial septum, and a vessel which passes through the sinus node, the "sinus node artery".[78] This sinus node artery can be regarded as a main source of blood to the region of the sinus node and also as a source of blood to certain specialized areas of the conduction system.

Starting from these dual functions, the pathologists must interpret the presence of obstructive changes in the sinus node artery as having complex consequences. In addition, he must investigate if branches to the sinus node are not present in the same coronary arterial tree along the course of the left circumflex artery. The functional significance of the presence of obstructive lesions in the sinus node vessel occurring as a branch of the right coronary artery may be associated with a long or short left circumflex artery in the atrioventricular sulcus, the degree of collateral vessel development in the walls of the atria and atrial septum, the origin of the atrioventricular node vessel and its luminal size, the possible presence of the descending septal branch, and the left Kugel's branch. The proportion of cases in which the sinus node artery is a branch of the right coronary artery varies from more than 40% to more than 60%. In about 30% of individuals, the sinus node artery originates only from the circumflex artery; it may also arise from the bronchial artery or directly from the descending thoracic aorta. Also of note is the observation that in more than 20% of cases a posterior sinus node artery can be detected, having a diameter up to 2 mm, similar to a major coronary artery. It may arise from the right coronary artery or from the left circumflex artery.[2]

In experimental models the sinus node branch was used to deliver drugs, hormones, and other chemical agents to the node.[79-81] Ligation of the sinus node artery has been shown to alter the rate of sinus node discharge. Since this vessel seems to contribute two thirds of the flow to the sinus node region, the effect of this ligation depends on the available collateral circulation. An abrupt increase in pressure in the sinus node artery may be followed by bradycardia, whereas acute embolism immediately shifts the pacemaker to a junctional focus, a change which also appears after various direct injuries to the sinus node. Finally, complete excision of the sinus node was followed by a return to an unstable atrial pacemaker.[82]

Many published data and our own experience show that a macro- and microscopic examination of the sinus node vessel is necessary, particularly in cases with conduction disturbances followed by sudden cardiac death. In two individuals who died suddenly and unexpectedly, occlusion of the vessels supplying the conduction system was the only significant finding at autopsy.[83] A study based on post-mortem coronary angiography and histological examination of the conduction system in persons who died suddenly and unexpectedly, revealed, in addition to a diffuse atherosclerotic involvement, the presence of obstructive lesions in the vessels supplying the conduction system.[84] We also found severe narrowing of the arteries supplying the conduction system in a patient with severe bradycardia, bigeminy, and atrioventricular block, followed by cardiac arrest. A second patient with more than 80% narrowing of the arteries supplying the conduction system manifested syncopal episodes, followed by chest pain and left bundle branch block; the patient died from ventricular fibrillation and did not show severe stenotic lesions in the major coronary arteries.[67] In a study on the small arteries of the heart, the following lesions were detected in the sinus node artery: fibromuscular dysplasia, intimal proliferation, primary medial

necrosis, medial dissection, fibrinoid necrosis, inflammation, emboli, thrombosis, and mural deposits. Particularly of note seems to be focal fibromuscular dysplasia, suggested to occur as an inherited monoclonal abnormality of smooth muscle cells.[85]

Investigations carried out in our laboratory showed that intimal thickening in the sinus node artery may be detected even in children aged 11 to 15 years; this thickened intima may obstruct more than 50% of the vessel lumen in young adults and up to 75% in mature adults. As concerns the first atherosclerotic plaques, they have been visualized for the first time as fibromuscular plaques in both male and female subjects aged 31 to 35 years.[64,65]

The descending septal vessel was present in 77% of cases in special investigations.[86] It appears as a branch of the proximal segment of the right coronary artery and supplies the region of the upper ventricular septum and the conduction system. A single middle or long descending septal vessel was found in 56% of hearts, whereas in 25%, it originates from a common trunk with the conus branch. It seems that this descending septal artery becomes more clearly visible in coronary beds with severe stenotic lesions and represents a further source of supply to the conduction system.

The right (acute) marginal artery, with a diameter of 0.5 to 2.5 mm, courses along the acute margin of the heart toward the apex. When a short right coronary artery exists, this branch may represent its distal segment. When a large right marginal branch is present, with a diameter similar to that of a major coronary artery, it usually gives rise to the posterior descending branch and to small left ventricular branches. In our material, some apparently healthy subjects who died in accidents showed stenotic atherosclerotic plaques in the proximal segment of the right marginal branch. The prevalence of plaques in subjects aged 46 to 50 years was 3%. Intimal thickening could be revealed starting from the 26 to 30 year old age group; in subjects aged 46 to 50 years, the ratio intimal thickness vs. medial thickness was 2.71. This very thick intima reduced the vessel lumen by about 50%.[65,67]

The atrioventricular node artery has a diameter of approximately 1 mm and in about 90% of cases arises at the level of the crux cordis from the right coronary artery, on the opposite side of the point of origin of the posterior descending artery. It appears closely related to the initial portion of the left ventricle, running through the fat-filled space located at the inferior wall of the right atrium to supply the atrioventricular node and the bundle of His. The pathologist can visualize this vessel at the level of the so-called "U-curve" of the right coronary artery. Detection of the left origin from the circumflex artery in approximately 10% of cases is more difficult than that of the right. Irrespective of its parent vessel, the atrioventricular node artery is connected with important collaterals from the first and second septal branches of the left anterior descending artery.

The first fibromuscular plaques were revealed in our material in the atrioventricular node artery in both male and female subjects aged 36 to 40 years. Only 2% of unselected cases 46 to 50 years old who died in accidents showed stenotic atherosclerotic plaques in the atrioventricular node artery, compared to 16% in selected cases who died of myocardial electrical disturbances (Figure 42). Total occlusion of this vessel was detected in patients with complete heart block followed by sudden cardiac death. This vessel also exhibited in our material a rapid onset of a very thick intima. In certain children aged 11 to 15 years the ratio of intimal thickness to medial thickness was 2.35.[65] This ratio rose to 3.11 in subjects aged 46 to 50 years, and this very thick intima reduced the vascular lumen up to 75% in undistended vessels. Likewise, in other reports, occlusion of the artery supplying the atrioventricular node appeared as the main pathologic finding in cases of sudden cardiac death.[83]

The presence of stenotic or occlusive plaques in the atrioventricular node artery, or the presence of very thick intimas, may be associated with areas of necrosis and/or fibrosis of the conduction tissue and such changes could be responsible for complete heart block or lethal arrhythmias.[87]

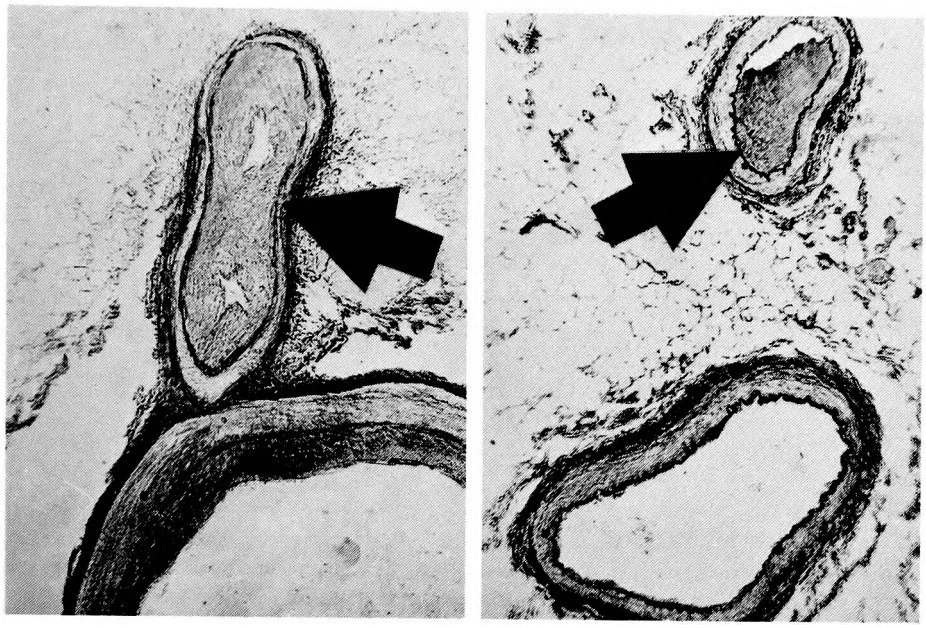


FIGURE 42. The arteries supplying the conduction system: at left, the sinus node vessel; at right, the atrioventricular node vessel both involved by grossly neglected obstructive lesions (arrows). The patient is 52-year-old woman with severe bradycardia, bigeminy, and atrioventricular block, followed by cardiac arrest. Absence of myocardial infarction and/or lumen compromising plaques in the major coronary arteries. (Left) Severe narrowing of the lumen of the artery supplying the sinus node, whereas the proximal segment of the right coronary artery shows only a moderate intimal thickening. (Right) Severe narrowing of the lumen of the branch supplying the atrioventricular node, whereas the intermediate segment of the right coronary artery did not exhibit pathologic changes. (From Velican, C. and Velican, D., *Atherosclerosis*, 47, 215, 1983. With permission.)

All observations on the relationships between the presence of obstructive lesions in the atrioventricular node artery and the presence of pathologic changes in the conduction tissue supplied by the respective vessel point to *a need for more frequent routine examination of the vessels supplying the conducting tissue and of the conducting tissue itself.* On the other hand, the pathologist must be aware of the many anatomical variants of both sinus node and atrioventricular node arteries and of the possible functional significance of these anatomical variants.[87-91] The available literature clearly shows that examination of the sinus node and atrioventricular node vessels has been particularly fruitful in the hands of many pathologists. Since the study of these vessels requires both macro- and microscopic examination, it seems necessary to ask when we must add histological studies to the naked eye observations. The answer could be "a compromise between the practically possible and the theoretically desirable".[92]

The posterior descending artery with a diameter of 1 to 3 mm is, in about 90% of cases, a major terminal branch of the right coronary artery, arising at or immediately before the level of the crux cordis, and coursing toward the apex in the posterior interventricular sulcus. In many cases, the posterior descending vessel may show a diameter similar to that of a major coronary artery and particularly of the left anterior descending artery. When the anterior descending artery was hypoplastic, the posterior descending artery had a diameter of up to 3.0 mm; this large vessel was usually distributed to the apical area of the left ventricle, to the inferior of diaphragmatic wall of both ventricles, and to the lateral wall of the left ventricle (Figure 43). When the right coronary artery was hypoplastic, the left anterior descending artery appeared very long and large, and the posterior descending vessel arose from the left circumflex artery. Finally, when the posterior descending artery was hypoplastic,

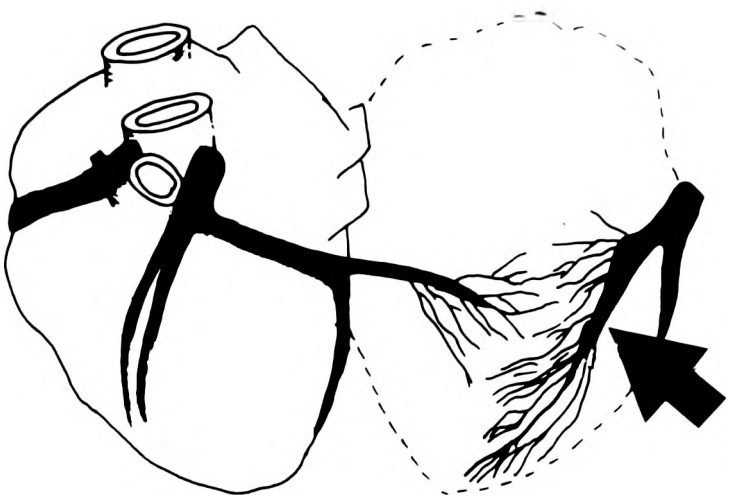


FIGURE 43. Posterior descending artery (arrow) showing a considerable development starting from childhood. This minor atherogenic deviation from the common type of distribution of the coronary arteries was detected in 4% of our unselected cases and was often associated with two short and narrow left anterior descending arteries. Consequently, the posterior descending artery supplied a large part of the crux cordis and of the apical area of the heart.

the anterior descending artery coursed around the apex and ascended to the area usually supplied by the posterior descending artery, particularly the posterior half of the interventricular septum. In our material, 13% of unselected subjects aged 46 to 50 years who died of accidental causes showed atherosclerotic plaques in the proximal segment of the posterior descending artery. The first plaques were detected in male and female subjects aged 21 to 25 years; in children and juveniles aged 11 to 15 years, an intima thicker than the underlying media was found.[64,65,67]

There is now fairly good evidence that gross inspection of the proximal segment of the posterior descending, first diagonal, first septal, right and left marginals, and of the vessels supplying the conduction system, associated with microscopic examination of the conduction system itself, may lead to considerable improvement of the clinico-pathologic correlations related to the natural history of coronary atherosclerosis (Table 4).

B. Examination of Coronary Atherosclerotic Involvement

1. General Observations

The need for better heart autopsy in various stages of the natural history of coronary atherosclerosis was emphasized by many pathologists and autopsy protocols devised specifically for this aim were proposed during the last 3 decades.[93-114]

Of particular value seems to be these two recommendations: "A pathologist must not report till histologic examination of the coronary tree is complete".[92] "Pathologists must be trained in anatomic pathologic subspecialities, since this subspecialization may improve the image of autopsy pathologist and provide more expert analysis".[115]

The examination of coronary atherosclerotic involvement by means of both gross inspection and light microscopy is not a trivial task. Long experience in this field allows us to assume that this examination is often associated with many difficulties:

1. There are difficulties in defining the anatomical branching pattern of the coronary arterial tree.
2. There are difficulties in delineating an age-related change of physiologic character from an age-related change which predisposes to atherosclerotic involvement, partic-

Table 4

NUMBER OF CORONARY ATHEROSCLEROTIC PLAQUES IN THE MAIN BRANCH VESSELS OF THE MAJOR CORONARY ARTERIES[a]

Main branch vessels	Age groups (years)													
	16—20		21—25		26—30		31—35		36—40		41—45		46—50	
	GI	LM	GI	LM	GI	LM	GI	LM	GI	LM	GI	LM	GI	LM
First diagonal artery	0	0	0	1	0	4	1	4	1	4	3	7	5	8
First septal artery	0	0	0	2	0	5	2	4	4	6	7	11	8	12
Left marginal artery	0	0	0	0	0	1	0	1	0	1	0	2	0	2
Sinus node artery	0	0	0	0	0	0	0	0	0	1	0	2	0	2
Right marginal artery	0	0	0	0	0	0	0	1	0	1	0	2	0	3
Atrioventricular node artery	0	0	0	0	0	0	0	0	0	0	0	1	0	2
Posterior descending artery	0	0	0	2	0	3	3	5	3	6	6	11	9	13

[a] This is revealed by gross inspection (GI) and light microscopy (LM) in 640 subjects aged 1 to 50 years who died in traffic accidents.

ularly what represents a "normal" intima in successive age groups from children to mature adults.

3. There are difficulties in identifying all types of early atherosclerotic lesions and all patterns of progression toward advanced lesions, and in identifying static, asymptomatic advanced lesions and dynamic advanced lesions of possible clinical significance.

Additional difficulties may be related to the existence of a neglected coronary atherosclerosis.[67] This neglected coronary atherosclerosis was delineated in our laboratory and includes:

1. Lesions visible only by light microscopy (fibromuscular plaques, small gelatinous lesions, small intimal necrotic areas, incorporated microthrombi, and intramural thrombi) and which are recorded on gross inspection as "normal intima"
2. Lesions visible both on gross inspection and light microscopy and which are present in coronary vessels which are not opened during routine autopsy and are not removed for light microscopic examination — posterior descending, first diagonal, first septal, left marginal, right marginal, sinus node, and atrioventricular node vessels[64,65]

Among the neglected lesions are those of the conduction system and of the nerves and ganglia of the heart. Although more than 50% of patients with coronary heart disease are victims of sudden cardiac death produced by ventricular fibrillation and other types of severe arrhythmias, very few pathologists consider it necessary to investigate the cardiac conduction system and its supplying vessels. We are firmly convinced, together with other investigators, that in many cases this light microscopic examination may be very fruitful, beginning with the pediatric age group.[83]

There are many methods which try to classify grossly visible atherosclerotic lesions in well-delineated groups according to selected criteria and to use a macroscopic grading in an attempt to quantitate coronary atherosclerosis.

The need for such methods was mainly due to the fact that in the middle 1950s, pathologists began to compare the degree of atherosclerotic involvement in two or more populations. The principal objective was to determine the influence of age, sex, race, environmental conditions, and of associated diseases on the natural history of coronary atherosclerosis and particularly on its end stages manifested as angina pectoris, myocardial infarction, or sudden cardiac death.

To compare the degree of atherosclerotic involvement in two or more populations, it is necessary to quantitate lesions, to use a macroscopical grading of atherosclerosis. Many methods to determine this macroscopical grading of atherosclerosis have been proposed and the most important are discussed in the next pages. The possibility for quantitating lesions allowed studies of the geographic pathology of atherosclerosis; moreover, they provided the opportunity to relate the extent and severity of lesions to environmental agents and clinical manifestations of coronary heart disease.[116] The macroscopic grading of atherosclerosis was also used to evaluate the extent and severity of atherosclerosis in particular arterial beds, to perform comparative studies between cerebral and coronary arteries involved by atherosclerotic lesions, or among different major coronary arteries.[45] Finally, an additional principal objective of attempts to quantitate coronary atherosclerosis was to demonstrate if a higher coronary heart disease mortality and morbidity rate in a population is associated with more extensive and severe atherosclerotic involvement in autopsied persons from that population. Of note are the attempts to use methods which quantitate coronary atherosclerosis to improve clinico-pathologic correlations in selected patients.

Both the percent intimal surface covered with atherosclerotic lesions and the degree of luminal narrowing produced by atherosclerotic lesions have been largely used as quantitative

methods by pathologists. In the proximal segment of the major coronary arteries (particularly in the left anterior descending and left circumflex arteries), the degree of luminal narrowing produced by atherosclerotic lesions can often be correlated with the severity of the surface involvement of these arteries. On the other hand, in the intermediate segment of the left anterior descending and circumflex arteries, as well as in many coronary branch vessels with a diameter of 1 to 2 mm, the severity of surface involvement is usually reduced, whereas the degree of luminal narrowing is very important. This is mainly due to the more reduced caliber of these vessels which can be markedly narrowed by thick localized plaques.

The data currently available indicate that the techniques which try to quantitate the percentage of intimal surface covered with nonraised and raised lesions are inadequate to evaluate the functional consequences of the stenotic atherosclerotic plaques.[117] The results obtained with such methods are not superposable on those furnished by in vivo coronary angiography. In addition, clinicians need precision in naming lesions, for names may suggest pathogenetic implications and therapeutic possibilities. The terms "stenotic" or "occlusive" lesions have a totally different significance for the clinician than the terms "nonraised" and "raised" lesions. A clinician can accept as a cause of sudden cardiac death the existence of obstructive lesions in the vessels supplying the conduction system of the heart, but he is unable to relate sudden death to an intimal surface one fourth or one third covered with raised lesions.

Many methods which try to quantitate coronary atherosclerosis chiefly rely on vessel surface sudanophilia, considered by some pathologists to be a hallmark of atherosclerotic involvement. There are investigators who assume that there is an actual risk of missing the very early lesions if we do not use either macroscopic fat staining techniques or frozen sections to identify the location of the fat. This may be true in laboratory animals submitted to cholesterol-rich diets, but human coronary atherosclerosis, a multifactorial disease, offers a complex picture of early lesions. They may occur as lipid-free fibromuscular plaques, lipid-free gelatinous lesions, lipid-free small intimal necrotic areas, lipid-free incorporated microthrombi, or intramural thrombi. All these early lesions are missed by the commonly used quantitative methods which rely only on sudanophilia and gross inspection.

The exclusive use of these quantitative methods which rely on macroscopic sudanophilia constrains the pathologist to accept the views that lipid is the hallmark of atherosclerosis, fatty streaks are the only early lesions, and fatty streaks being the only early lesions are also the only possible precursor of advanced lesions occurring as fibrous plaques. These methods also constrain the pathologist to accept the view that beside the fatty streak and noncomplicated or complicated fibrous plaques, there is normal intima.

The existence of this normal intima in mature adults and elderly people and particularly in patients who died from coronary heart disease was not specifically demonstrated by any of the authors, who assumed that in subjects with 20 or 30% of the intimal surface covered with nonraised and raised lesions, 70 or 80% is normal intima. The truth is probably considered self-evident and requires no additional investigations. In our material, many areas of macroscopically normal intima examined by light microscopy appeared as pathologic regions of the vessel wall, being diffusely involved by atherosclerosis (Figure 44). We agree with the pathologists who have revealed that particularly in subjects who died of coronary heart disease, "all portions of the extramural coronary arteries are involved by atherosclerotic lesions".[118,119] Both successive longitudinal sections or serial cross-sections clearly demonstrate that in subjects more than 50 years old it is very difficult to find areas of normal intima. In addition, even the term "normal intima", as used in the methods which try to quantitate coronary atherosclerosis, requires reconsideration.

As already stated, the visible lesions in many cases may represent that portion of the iceberg which peeks above the surface. The great mass of the iceberg, namely the diffuse atherosclerotic involvement (also including normal intima), go undetected.

Our own experience shows that the pathologist's main handicap when trying to quantitate

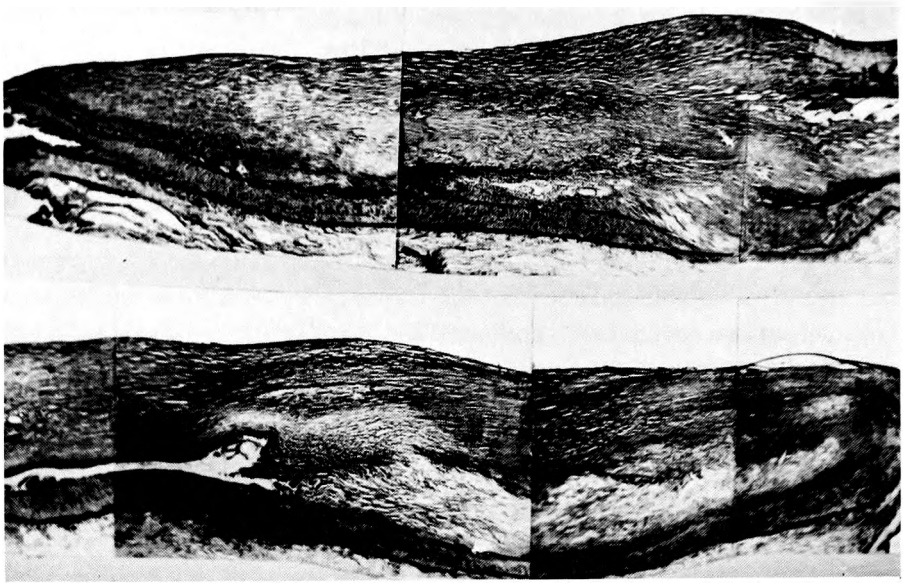


FIGURE 44. An unbranched, 1.5-cm long segment of the left anterior descending artery between the branching points of the first and second diagonal vessels. On gross inspection we recorded neither fatty streaks nor raised fibrous plaques; consequently, this segment might be theoretically considered to represent "normal intima". On light microscopic examination a diffuse, uninterrupted severe atherosclerotic involvement appeared on successive longitudinally cut sections. (Resorcin fuchsin-alcian blue. Magnification × 80.)

coronary intimal surface involved with atherosclerotic lesions, does not reside in his ability to reveal intimal sudanophilia and the raised character of some lesions, but in:

1. The limitations of the methods commonly used in detecting all types of early lesions (fibromuscular plaques, gelatinous lesions, intimal necrotic areas, incorporated microthrombi, intramural thrombi, fatty streaks)
2. The variability of physiologic or pathologic changes which may occur as sudanophilic lesions
3. The reduced possibility of gross inspection to detect other pathologic changes except those appearing as fatty streaks and noncomplicated and complicated fibrous plaques
4. The inability of gross inspection to reveal, in longitudinally opened vessels, lesions located in the basal intima
5. The existence of atherosclerotic lesions, some of certain clinical significance, in arterial segments which are not opened longitudinally during routine autopsy (Figures 45 and 46)[64,67]

In our hands, the methods used to measure the percentage of intima surface covered with atherosclerotic lesions gave systematically unsatisfactory results. In children, adolescents, and young adults, fibromuscular plaques, gelatinous lesions, intimal necrotic areas, incorporated microthrombi, and intramural thrombi were neglected (Table 5). The diffuse character of atherosclerotic involvement was also neglected as illustrated in Figure 44.

Some investigators continue to expend considerable effort in an attempt to offer cheap, clean, rapid, and simple measurement systems. A commercial Xerox machine, in which the opened artery can be xerographed, may appear well suited for this purpose. Unfortunately, it would also be necessary to have an additional machine to indicate which of the so-called fatty streaks are indeed lesions and which are only reversible accumulations of lipid-filled macrophages acting as a physiological lipid-clearance system; to indicate the proportion and

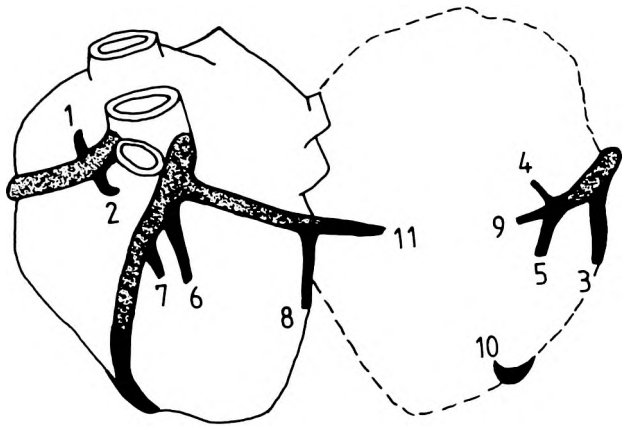


FIGURE 45. Schematic presentation of the major coronary arteries and of their main branch vessels. In gray, the vascular segments longitudinally opened during routine autopsy. In black are the vascular segments overlooked by many pathologists and which may include atherosclerotic lesions of possible clinical significance: 1, conus; 2, sinus node; 3, right marginal; 4, atrioventricular; 5, posterior descending; 6, first diagonal; 7, first septal; 8, left marginal branches. Also in black: 9, terminal segments of the right coronary artery; 10, left anterior descending artery; and 11, left circumflex artery.

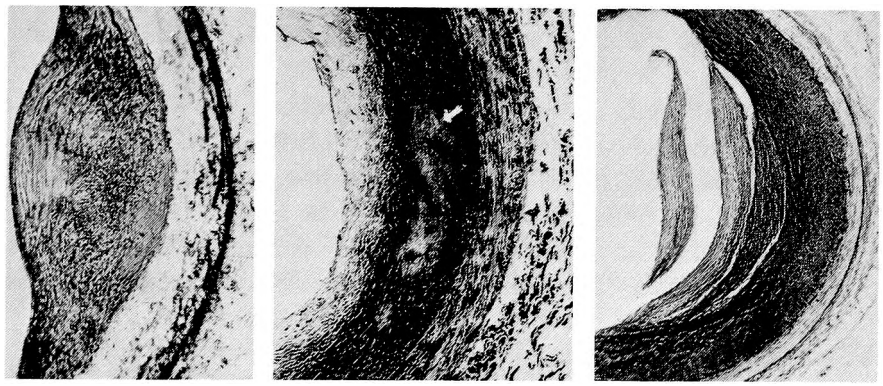


FIGURE 46. Early lesions which represent the main morphological substratum of the grossly neglected coronary atherosclerosis: the fibromuscular plaque (left), the gelatinous lesion or intimal necrotic area (middle, arrow), and the incorporated microthrombi (right). (From Velican, C. and Velican, D., *Atherosclerosis*, 47, 215, 1983. With permission.)

type of atherosclerotic lesions which cannot be detected grossly after Sudan staining; to reveal the proportion of lesions of possible clinical significance among raised lesions; and to estimate the degree of luminal narrowing and the adequacy of collateral circulation.

Human coronary atherosclerosis is not measured in importance by its amount of fatty streaks and raised lesions, but by its potential to produce myocardial clinical manifestations. These myocardial clinical manifestations are strongly related to the facility with which blood passes through the whole coronary arterial tree and not only through the proximal segment of the major coronary arteries. *The morbidity and mortality from myocardial infarction and sudden cardiac death can more easily be explained by progressive luminal narrowing to a point at which ischemia occurs in the absence of an adequate collateral circulation than by a sum of focal lesions which involve the intimal surface of the proximal segment of the major coronary arteries.*

Table 5
PERCENT OF SUBJECTS 1—50 YEARS WITH GELATINOUS LESIONS (GL), INTIMAL NECROTIC AREAS (NA), INCORPORATED MICROTHROMBI (IM), AND INTRAURAL THROMBI (im)

Age groups (years)	GL and NA	IM and im
1—5	0	1
6—10	2	1
11—15	6	2
16—20	14	8
21—25	32	10
26—30	56	14
31—35	72	18
36—40	84	22
41—45	95	28
46—50	100	48

[a] Recorded by gross inspection as "normal intima", but revealed by light microscopy.

There is no substitute for rigorous macro- and microscopic examination of the whole length and all segments of the coronary arterial tree and of the conduction tissue and its supplying vessels.

As long as (1) the significance of fatty streaks remains obscure and we continue to overlook other early lesions and the prevalent development of atherosclerotic plaques on their own, without precursors; (2) we continue to overlook the complex pathology of coronary branch vessels and particularly, the obstructive character of some of their lesions; and (3) as long as we are unable to correlate the intimal surface area covered by lesions with the real extent of lesions in the basal intima, certain of our measurements might represent mere amusements.

2. Measurement of the Atherosclerotic Intimal Surface

We do not intend to present and discuss the multitude of simple to fairly sophisticated instrumentation or mathematical models to objectively quantitate the coronary atherosclerotic involvement, but only to give some demonstrative examples in chronological order.

The first important attempt to offer an objective method of recording necropsy observations was published 3 decades ago.[93] This procedure was considered easy to apply, avoiding undue demands upon the time and resources of the pathologist. It is limited to gross inspection and requires nothing but systematic observation, estimation, and recording. The types of lesions proposed to be recorded are classed in four grades of atherosclerotic involvement: grade 1, lipid streaks, spots, and patches occurring as very superficial, thin yellow, subendothelial accumulations which may just perceptibly elevate the intimal surface; grade 2, elevated, smooth-surface, fibrous plaques of variable lipid content; grade 3, plaques with ulcerations, necrosis, and hemorrhage; grade 4, calcified plaques. These four successive grades of coronary atherosclerotic involvement may reflect an oversimplified view of the natural history of the disease. The extent of atherosclerotic involvement was considered negligible when the proportion of intima covered with lesions was less than 5%, minimal between 6 and 15%, mild between 16 and 33%, moderate between 34 and 50%, and severe

at more than 50%. Of particular interest is the idea of obtaining an index of luminal narrowing, a method able to calculate an "atherosclerotic index" graded from 0 to 100 (semiquantitative data on the extent of atherosclerotic involvement and on the prevalence of a particular type of lesion, as well as the degree of vessel narrowing).

Beginning with the 1950s, a new orientation became prevalent in the field of human atherosclerosis research, related to the geographic pathology of the disease. Two eminent pathologists, Strong and McGill, together with the team of investigators from Louisiana State University (New Orleans), opened this new line of research. They offered a personal view on the natural history of coronary atherosclerosis, their results demonstrating that this disease is the most significant factor in determining the risk of myocardial clinical manifestations in a population. They also showed that coronary atherosclerosis may develop early in life, 20 years or even more before the onset of the myocardial clinical manifestations.[98]

The study of the geographic pathology of coronary atherosclerosis was further stimulated by the method proposed by the World Health Organization Study Group on Classification of Atherosclerotic Lesions.[120] This method tried to draw the pathologists' attention to the importance of macroscopical grading of lesions; it combines the proportion of areas showing sudanophilia with those with fibrotic and calcified lesions. In the evaluation of atherosclerosis, the percentage of total involvement is obtained by visual observation and subjective estimation of the intimal surface covered with lesions. A numerical profile for each vascular segment can be obtained in which the percentage of total involvement is given first and then the percentage of each type of lesion is considered. Although these observations are subjective, the agreement between observers was usually very good. The WHO study group defined the term "fatty streak" or "spot", a superficial yellow or yellowish-gray intimal lesion which stains selectively by fat stains. This term delineates a naked-eye description of lipid-rich superficial intimal change. The classification of atherosclerotic lesions covered by WHO does not imply a pathogenetic sequence and can be used as a descriptive one. This method could be improved by preserving arterial specimens in transparent plastic bags.[94] This has greatly facilitated the accumulation of statistical data on the natural history of coronary atherosclerosis and has stimulated interest in developing new methods for grading atherosclerotic lesions.

Many pathologists showed enthusiasm for the use of these quantitative methods, whereas others considered that "a macroscopical grading of atherosclerosis in an autopsy series, performed routinely by post-mortem examiner, has hardly any scientific value".[11] It should therefore be abandoned as the sole basis for analysis of atherosclerosis.[121] Similar pessimistic views resulted from comparisons between visual estimation and planimetry. If planimetry is considered an objective method, then the inaccuracy of the visual assessment becomes very clear. The error is considered certain to increase when subdivision into different types of lesions is attempted.[122]

Perusal of the literature clearly demonstrates that the believers continue to believe and the nonbelievers not.

A more objective method was proposed for grading atherosclerotic lesions which is based on an optical-electronic analysis of photographic and radiographic transparencies prepared from arteries.[123] The results obtained were considered significantly more reliable than those furnished by subjective visual estimation. An additional contribution to obtain a more objective quantitation of atherosclerosis on necropsy material is represented by a method using planimetry and point counting.[124]

In a very important study on the geographic pathology of atherosclerosis,[125] the coronary arteries were opened lengthwise with blunt, pointed iris scissors, fixed in 10% formalin for 24 hr or longer and stained with sudan IV. Lesions were evaluated while the coronary arteries were in plastic bags. Specimens were identified with code numbers, so that graders had no knowledge of age, race, sex, and cause of death. Each of the major coronary arteries was

graded separately, the left main coronary artery together with the left anterior descending artery. For each major artery, the total percentage of the intimal surface affected by all types of atherosclerotic lesions was estimated and the proportion contributed by each of the four types of grossly distinguishable lesions was recorded. The final value of coronary lesions for each case was derived by averaging the values for each of the three major coronary arteries. Information on age, sex, race, cause of death, and other pertinent clinical data were coded, transferred to punch cards, and analyzed on a Digital computer.[98,101] This method allowed the measure of the distribution of fatty streaks and of noncomplicated and complicated fibrous plaques in several countries, in order to relate the extent of these lesions to environmental changes and agents, race, sex, and particularly to the clinical manifestations of coronary heart disease.[125] The relation between the severity of intimal surface involvement and myocardial clinical manifestations was judged without any reference to the stenotic or occlusive character of plaques, or to the possible presence of occlusive lesions in vessels not opened during autopsy or in branch vessels which were systematically eliminated. In a recent work, attempts wee made to introduce luminal narrowing as a parameter able to increase the sensitivity of the method, starting from the largely accepted view that quantitation of the luminal narrowing is essential in separating cases with normal from cases with abnormal coronary blood flow. In one such study the coronary arteries were first opened longitudinally and stenoses were defined as a reduction of the luminal diameter by more than 40%, judged visually by one of the authors.[126] The arteries were then stained with sudan IV and sealed in plastic bags and a team of specially trained pathologists graded the specimens for involvement with fatty streaks and raised lesions.[126,127]

Quantitative data were also obtained using the polar coordinate method of quantifying lesions at the ostium.[128] The vessels are opened longitudinally, fixed, and processed in saturated sudanophil stain to demonstrate lipid accumulation. The centroid of the ostium is placed at the polar coordinate origin and oriented so that it corresponds to a known anatomical or physiological landmark such as blood flow direction. The ostium is traced, the distance between the ostium lip and the edge of the lesion is measured at different radial angles, and the data are plotted on rectangular coordinates. This method, which is easily computerized, is considered adequate to quantitate specific ostial lesions and to produce mean composite images representative of an entire population. An additional method has been proposed to record the topographic distribution of atherosclerotic lesions in "unambiguous statistical terms". It could provide the information necessary for the development of a rational sampling strategy for the study of the localizing factors and of their role in atherogenesis.[111]

An investigation of particular interest is the association of data recorded from longitudinally opened arteries with those obtained from post-mortem angiography and casts.[129] The intimal surface involved with lesions, the percent reduction in lumen, and the length of the stenosed artery in longitudinally opened vessels, in post-mortem angiograms, in casts, and in the three media together, was estimated. Artery angiogram, together with casts, seem indicated when clinico-pathological correlation is the purpose of the investigation.

3. Measurement of the Narrowed Lumen

A new line of atherosclerosis research recommended by WHO 3 decades ago is based on the post-mortem evaluation of the degree of luminal narrowing.[130] This orientation preceded the introduction of coronary angiography a method which also evaluates the degree of luminal narrowing in the coronary arteries but in living patients. Since the degree of encroachement of atherosclerotic plaques could be determined both in vivo and in post-mortem material, a considerable improvement in clinico-pathologic correlations in coronary heart disease was obtained.

An interesting coding system was proposed which indicates both the surface involvement by atherosclerotic lesions and the degree of luminal insufficiency produced by the respective

lesions.[100] According to this method, grade 1 severity of atherosclerosis is attributed to lesions affecting only a small part of the vessel circumference, with no lumen obstruction. Grade 2 severity codes a diffuse, thin plaque that does not involve the entire vessel circumference with minimal lumen narrowing, or a small thick plaque that produces less than 25% lumen reduction. Grade 3 severity is characterized by a diffuse, thin plaque involving the entire circumference with mild luminal narrowing, or a localized thick plaque producing 25 to 30% lumen obstruction. Finally, grade 4 severity includes a thick plaque developed along the entire vessel circumference with moderate or marked lumen narrowing, or a localized plaque resulting in over 50% lumen obstruction.

During the last 20 years, the cross-sectional intimal area and percent stenosis became gradually common morphological descriptors of coronary atherosclerosis. Cross-sectional intimal areas, i.e., plaque areas, describes lesion size, whereas percent stenosis describes the degree of luminal insufficiency produced by plaques. Both can be obtained by very careful examination of the extramural segments of the coronary arteries by laminating the unopened vessels into thin slices about 2 to 3 mm thick. Such examination can be associated with various estimates of ventricular changes, gross and histologic grading of coronary atherosclerosis, gross and histologic mapping of lesions on complete cross-sections of the heart at several levels, and with studies on the conduction system.[103] These coordinated methods, which may be used sequentially are adequate for investigating the relationship between stenotic and occlusive lesions on the one hand, and myocardial damage, on the other hand.

A method which permits evaluation of the extent, location, and topographic characteristics of the stenosed segments consists in dissection of the coronary arteries together with the epicardial adjacent and as far as possible distally, fat in the fresh heart from the ostium. Branches of particular interest can easily be included in the preparation. The arteries are fixed overnight in a buffer formalin solution, the excess of epicardial fat being then easily removed without risk of inadvertently tearing the friable coronary arteries. If necessary, the arteries can be decalcified in 5% nitric acid for 24 hr, neutralized in a solution of lithium carbonate for 30 min, and washed for 1 hr in running water. After this, the coronary arteries are cross-sectioned at 2 mm intervals. The severity of luminal narrowing is then recorded, usually as the percentage reduction of the cross-sectional area of the lumen as compared to the normal lumen. Grids were used for comparison and certain results suggested that failure to use these grids resulted in underestimation of the reduction of the cross-sectional lumen, especially in cases of circular concentric plaques.[104] The degree of coronary artery stenosis can be related to age, sex, race, cause of death, prior history of myocardial clinical manifestation, environmental risk factors, etc.

An autopsy protocol to be used in patients with ischemic heart disease recommends fixation in formalin of the epicardial parts of the coronary arteries. The vessels are excised unopened with surrounding epicardial fat and little underlying myocardium, and are cross-sectioned at 6 to 8 mm intervals; the segments are serially numbered, and the proximal face of each segment is marked with India ink as a guide to correct embedding in paraffin. Right or left dominance is estimated and a record is made of which segments of the major coronary arteries give rise to important branch vessels (diagonal, septal, right marginal, posterior descending, left marginal, sinus node). These branch vessels are also serially sectioned, numbered, and labeled before embedding in paraffin. Each individual segment cross-sectioned 6 to 8 mm was then serially sectioned at 2 mm and the luminal narrowing was graded as follows: less than 25%, between 25 and 50%, between 50 and 75%, and more than 75% — acute and old occlusive lesions were also recorded. In addition to the ventricular walls, the conduction system was also investigated, particularly the sinus node and the atrioventricular node.[131]

In a study of coronary arterial narrowing at necropsy in acute transmural myocardial

infarction,[132] the hearts were fixed for at least 1 day in formalin. The four major coronary arteries were excised intact, X-rayed, and fixed for at least 1 day more; then each artery was cut across the longitudinal axis into approximately 5 mm segments and each segment was labeled sequentially. The segments were embedded in paraffin, stained by Movat's technique, and the luminal narrowing determined. The degree of narrowing was evaluated based on histologic examination of each cross-section magnified 25 to 30 times. A 95% agreement was found between the visual estimation of the percentage of cross-sectional area narrowing and that found by planimetry. This method demonstrated that coronary atherosclerosis among patients with fatal transmural acute myocardial infarction is a *diffuse process involving all major epicardial coronary arteries.*[132]

The maximum degree of cross-sectional area narrowing by atherosclerotic plaques was also determined by means of a video-based computer-linked system.[133] Coronary artery sections stained by Movat's technique were positioned on the stage of a projection light microscope. The image was then magnified × 650 onto opaque white paper and a tracing was made of the original lumen of the artery (denoted by the black-staining internal elastic membrane), the area occupied by the atherosclerotic plaque, and the area occupied by the thrombus. The percentage of cross-sectional area narrowed by atherosclerotic plaques and by thrombi and the residual lumen were then calculated. The most promising aspect of this complex approach to the coronary arteries and the heart is the potential correlation of functional, hemodynamic, electrophysiologic, and angiographic data with post-mortem findings. These complex methods allowed the revelation, for instance, of more severe coronary arterial narrowings in patients with type IV than in those with type II hyperlipoproteinemia. They reveal, likewise, that the percentage of 5 mm coronary segments narrowed 76 to 100% in the cross-sectional area by the atherosclerotic plaques in patients with type IV hyperlipoproteinemia (67%) exceeded that found in sudden cardiac death (36%), unstable angina pectoris (47%), and acute transmural infarction (34%); also of note is the observation that two or more of the major coronary arteries were narrowed severely in the cross-sectional area by atherosclerotic plaques in 96% of patients with types II and IV hyperlipoproteinemia.[134]

In a study of a series of coronary and violent deaths, the degree of coronary narrowing was classified according to its severity and also according to the number of sites obstructed using scores of 0 to 5. Score 0, no stenosis; score 1, less than 50% stenosis; score 2, 50% stenosis at one site, but no occlusion; score 3, 50% stenosis at more than one site, but no occlusion; score 4, almost total or total occlusion at one site; score 5, almost total or total occlusion at more than one site in an artery, or occlusion of the artery for a distance of several centimeters.[135]

In an investigation carried out on patients with coronary heart disease who died suddenly, a different method was used. The unopened hearts were inspected and roentgenographed, then the coronary arteries were cannulated and sequentially injected with green (left) and red (right) barium sulfate gelatin mixture at pressure of about 120 mmHg. The heart was roentgenographed after each injection. After the mixture had set, the arteries were dissected free of the myocardium, roentgenographed, opened longitudinally, cleaned, fixed in a flattened position in 10% buffered formaldehyde solution, stained with sudan IV, and sealed in plastic. For histologic examination, tissue from 12 standardized areas was embedded in paraffin and stained with hematoxylin-eosin and Gomori's trichrome-aldehyde fuchsin. Postmortem angiograms were examined together with the cross-section diameter of the coronary arteries for evaluating stenosis and occlusion. Using this method, occlusion of one or more coronary arteries was found in 45 to 70% of the coronary heart disease group who died suddenly.[136] In an additional study focused on the pathology of sudden cardiac death, the same methodology was used.[137]

Certain methods try to transform what was previously a "subjective analysis of data" to one which is considered to be "totally objective".[138] This transformation is based on a

quantitative analysis and statistical treatment of morphological results, permitting detection of subtle changes in the progression of lesions. For quantitative morphological evaluation of atheroma-like lesions, a computerized operator-interactive system for the measurement of lesions was used. This system requires a 64 K microcomputer, a high resolution digitizer tablet, dual floppy disc drives, and a videomonitor. In another investigation, a planimeter with a picture analysis OA-I calculator equipped with a digitizer was employed.[112] All of the three major coronary arteries were cut, *in situ*, transversely at the approximately 2-mm intervals from the orifice of the aorta to the smallest vessels that could be followed with the naked eye. The segments with maximal stenosis were removed for histologic examination, the degree of luminal narrowing being determined by measuring the narrowed lumen and the area bordered by the internal elastic membrane. Percent reduction of the lumen was calculated and the narrowing was expressed on a five-point scale as a stenotic score: 1, 0 to 24% stenosis of the cross-sectional area; 2, 25 to 49% stenosis; 3, 50 to 74% stenosis, 4, 75 to 99% stenosis; and 5, total occlusion. In addition, the stenotic index was calculated by adding each stenotic score of the three major coronary arteries together, giving a total grade for each heart. Stenotic index was evaluated by seven selected variables; age at death, gender, blood pressure, serum total cholesterol, body mass index, alcohol intake, and cigarette smoking,[112]

In the great majority of studies based on examination of the arterial cross-sections, severe atherosclerotic involvement was defined as 75% or greater stenosis, without any reference to the development of the collateral circulation. Theoretically, the use of all methods which refer to the degree of luminal narrowing requires adequate data on the development of collateral vessels able to bypass the respective narrowing. Practically speaking, these data are not available and many pathologists present the coronary circulation as end data.

A 75% cross-sectional area narrowing in undistended post-mortem coronary specimens seems to be equivalent to a 50% diameter reduction on the antemortem coronary angiogram.[139] Studies published by the Pathology Branch of the NHLB Institute indicate that patients with fatal coronary heart disease usually present at necropsy, narrowing by atherosclerotic plaques greater than 75%, in the cross-sectional areas of at least two of the three major coronary arteries.[140] They also show that over 30% of the entire length of the three major coronary arteries are narrowed to this extent.[119]

The methods which try to quantitate the degree of luminal narrowing of the coronary arteries produced by atherosclerotic plaques have also been used to reveal some trends in the severity of atherosclerotic involvement. In such a study the major extramural coronary arteries were examined by transverse sectioning at 5 mm intervals. The right coronary artery, left anterior descending artery, and left circumflex artery were graded in ascending order of reduction in cross-sectional luminal area by visual inspection by each prosector at the time of autopsy as follows: grade 1, up to 25% reduction in luminal area; grade 2, between 26 to 50%; grade 3, between 51 to 75%; grade 4, between 76 to 99%; grade 5, complete luminal occlusion. Grades 4 and 5 were designated as significant coronary disease. The use of this method revealed that the percentage of people with significant coronary disease increased during the period 1950 to 1969 and remained high in the 1970s.[141] On the other hand, a study based on the quantitation of the intimal surface covered with fatty streaks and noncomplicated and complicated fibrous plaques showed a decrease in the prevalence of atherosclerotic involvement.[142]

In the Juvenile Atherosclerosis Project,[143] subjects less than 10 years old were investigated, segments 2 to 4 mm thick being cross-sectioned and sampled near the origin of each major coronary artery. In subjects more than 10 years old, the major coronary arteries were cross-sectioned at 3 mm intervals along their whole course and the first 15 3 mm segments were processed for histologic examination. The total area of the thickened intima was measured by a polar planimeter on the histologic imaging projected on a screen. The maximal width

of the thickening was also measured directly by an ocular micrometer. The circumferential localization and extension of the thickening was referred to by dividing the arterial wall in four parts: anterior, lateral-right (or superior), lateral-left (or inferior), and posterior, according to the anatomical position of the vessel. New formation and proliferation of connective tissue fibers, smooth muscle cells, as well as accumulation of ground substance, lipid, fibrous tissue, necrotic tissue, and calcium, were expressed in the percentage of the total area of the thickened intima. The degree of luminal reduction was calculated by measuring with a polar planimeter the residual luminal area, referred to as percent of the normal lumen and by measuring the length of the stenosis in the sequential 3 mm segments.

An intriguing problem in all studies related to measurement of the narrowed lumen seems to be the significance of the changes produced by fixation and processing.[144] Moreover, disproportionate dimensional changes could occur during the successive stages of atherosclerotic involvement. In vessels with minimal narrowing by atherosclerotic plaques, fixation and processing may produce a decrease in total vessel and luminal cross-sectional areas, whereas absolute wall area (total vessel cross-sectional area − luminal cross-sectional area) does not change. When a moderate to severe atherosclerotic involvement exists, both the total cross-sectional area and wall area may decrease after fixation and processing, but the luminal area does not change. After processing for light microscopy, luminal size may be underestimated in segments with a mild atherosclerotic involvement, while in segments with a moderate to severe atherosclerotic involvement, luminal size could be overestimated.[144]

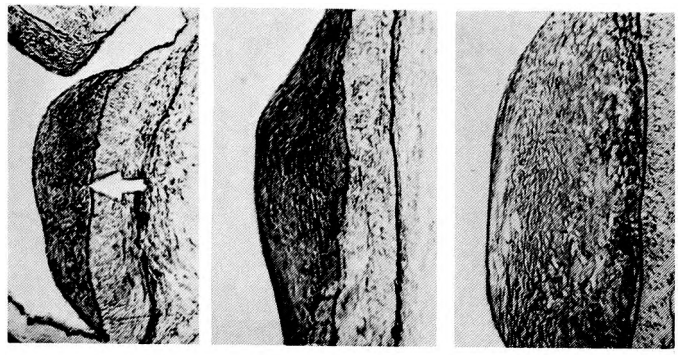
4. Comparative Evaluation of Similar Topographic Sites

In an effort to obtain an indirect sequence of the pattern and rate of progression of each type of atherosclerotic lesion from children to mature adults, a method was widely used in our laboratory based on macro- and microscopic visualization of similar topographic sites placed in sequence according to age, sex, cause of death, and branching pattern. Some illustrative examples are present in Figures 47 and 48.

Since we could not examine the same atherosclerotic lesion more than once, we observed thousands of static pictures in an attempt to obtain a "moving" picture. This indirect method, which includes an element of interpretation, was also used to reveal the influence exerted by some endo- and exogeneous risk factors for coronary heart disease on the pattern and rate of progression of atherosclerotic lesions.

In a study of the progression of coronary atherosclerosis from adolescents to mature adults,[145] ten selected topographic sites of the coronary arterial tree were placed in sequence according to age, sex, branching pattern, and smoking habit. The dynamic reconstruction (Table 6) revealed the existence of an age-related rate of progression of fibromuscular plaques, gelatinous lesions, intimal necrotic areas, fatty streaks, incorporated microthrombi, and intramural thrombi. This age-dependent rate of progression was interfered with by the action of risk factors which induced peculiar cycles of evolution of lesions, the prevalent change being an accelerated rate of progression toward advanced plaques of possible clinical significance. Table 7 demonstrates the results obtained using the above-mentioned method as concerns atherosclerotic plaques. These results show the existence of a cycle of evolution related to age from fibromuscular to mucoid; mucoid to lipid-rich; and fibromuscular, mucoid, and lipid-rich plaques to fibrohyaline and fibronecrotic plaques. This cycle of evolution was associated with a progressive luminal narrowing and a progressive lipid accumulation within plaques. Table 7 also shows that the fibromuscular plaques exhibit a similar prevalence in subjects ages 24 to 50 years, in spite of an important occurrence within this period of life of fibronecrotic and fibrohyaline plaques. This indicates that only a limited number of the fibromuscular plaques which exist in an individual are converted to advanced lesions with aging.

Table 8 summarizes our findings with respect to the influence of the anatomical branching



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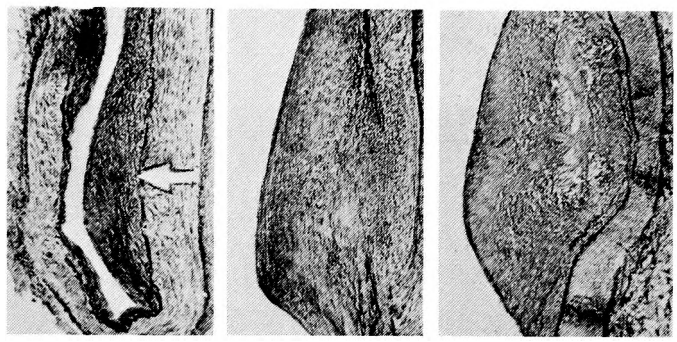
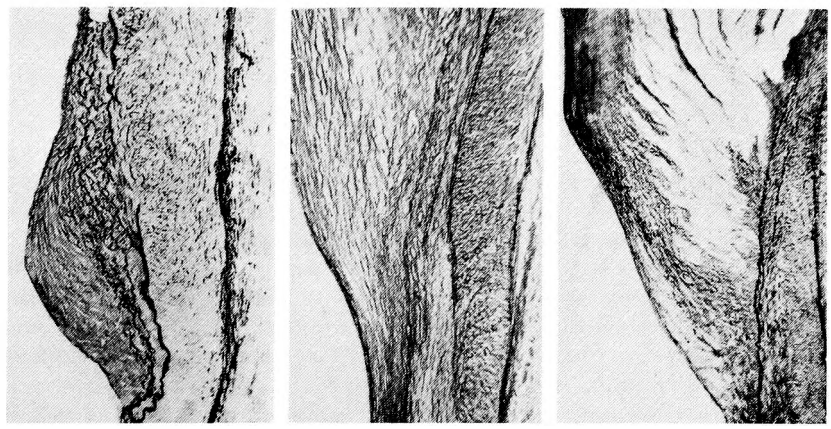


B



C

FIGURE 47. Similar topographic sites of the coronary arterial tree placed in sequence according to age. (A) The left circumflex artery at the branching point of the left marginal branch in three cases 16 (left), 28 (middle), and 49 (right) years old. Suggested transition from a fibromuscular (arrow) to a fibronecrotic plaque. (B) The right coronary artery at the branching point of the right marginal branch in three cases 22 (left), 41 (middle), and 54 (right) years old. Suggested transition from a fibromuscular (arrow) to a fibronecrotic plaque. (C) The left anterior descending artery at the branching point of the first diagonal vessel in three cases 8 (left), 32 (middle), and 52 (right) years old. Suggested transition from a fibromuscular to a fibronecrotic plaque. (D) The right coronary artery at the branching point of the right marginal vessel in three cases 18 (left), 39 (middle), and 47 (right) years old. Suggested progression from branch pads or cushions to fibromuscular plaques, and from fibromuscular to fibrohyaline plaques. (From Velican, C. and Velican, D., *Atherosclerosis,* 54, 333, 1985. With permission.)

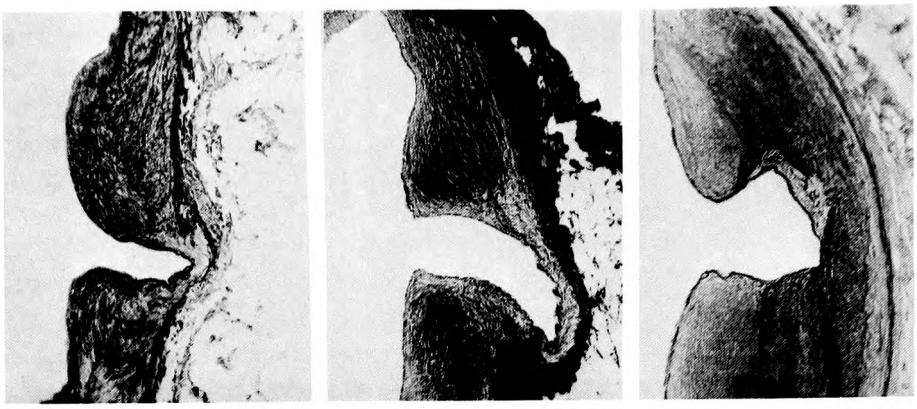
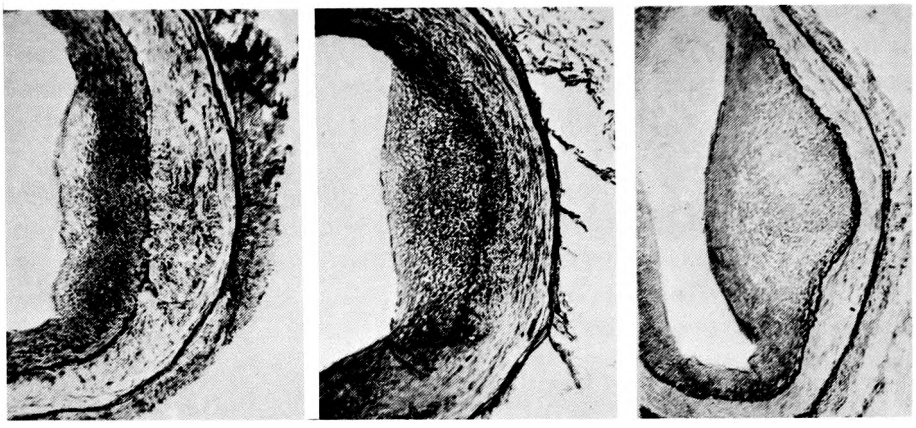


FIGURE 47D.



A

FIGURE 48. Similar topographic sites of the coronary arterial tree placed in sequence according to age. Nonbranched regions: (A) The proximal segment of the right coronary artery at 2 cm distal to the aortic origin in three cases 19 (left), 26 (middle), and 38 (right) years old. Suggested transition from a mucoid to a foam cell-rich and necrotic plaque. (B) The proximal segment of the left circumflex artery at 1 cm distal to the bifurcation area of the left main coronary artery in three cases 18 (left), 38 (middle), and 56 (right) years old. Suggested progression from a subendothelial area of insudation (arrow) to a necrotic and then a fibronecrotic plaque. (C) The proximal segment of the left anterior descending artery at 1 cm distal to the bifurcation of the left main coronary artery in three cases 14 (left), 33 (middle), and 48 (right) years old. Suggested transition from a very thick intima to an intima with areas of insudation (arrow) acquiring the light microscopic feature of a gelatinous lesion and then to a fibronecrotic plaque. (D) The proximal segment of the left anterior descending artery at 2 cm distal to the bifurcation of the left main coronary artery in three cases 22 (left), 28 (middle), and 46 (right) years old. Suggested progression from isolated foci of intimal necrotic areas to a necrotic and fibronecrotic plaque. (Resorcin fuchsin-alcian blue. Magnification × 120.)

pattern on the rate of progression of atherosclerotic lesions from adolescents to mature adults. This table shows that there were more numerous selected topographic sites with atherosclerotic plaques and intimal necrotic areas in subjects with than without atherogenic deviations from the common type of distribution of the coronary arteries. These differences appeared statistically significant for atherosclerotic plaques and intimal necrotic areas, but not for incorporated microthrombi and fatty streaks.[145]

In essence, the use of a method which allows comparative evaluation of similar topographic

FIGURE 48B.

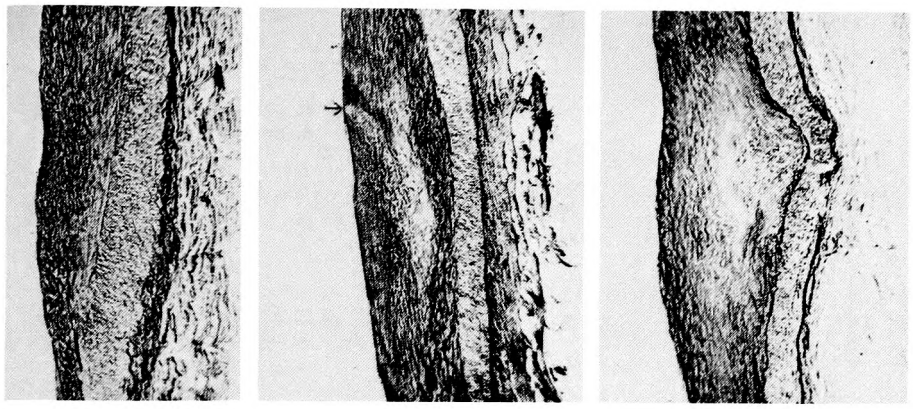


FIGURE 48C.

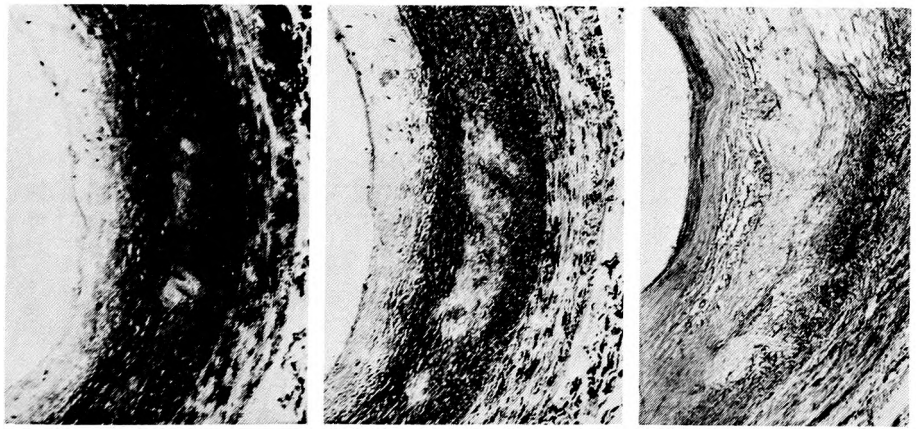


FIGURE 48D.

Table 6
AGE- AND SEX-RELATED RATE OF DIMINUTION (↓) OF NORMAL ASPECTS AND OF AUGMENTATION (↑) OF PATHOLOGICAL ASPECTS

		Age groups (years)										General trend in 25 years	
		21—25		26—30		31—35		36—40		41—45			
		↑	↓	↑	↓	↑	↓	↑	↓	↑	↓	↑	↓
Normal aspects													
Branch pads or cushions	T		1.41		1.73		1.36		1.09		1.02		3.66
	M		1.41		1.88		1.24		1.16		1.04		3.83
	F		1.42		1.62		1.44		1.05		1.00		3.50
Thickened intimas	T		1.27		1.27		1.15		1.27		1.37		3.37
	M		1.27		1.38		1.18		1.40		1.37		4.05
	F		1.27		1.19		1.12		1.13		1.37		2.69
Pathological aspects													
Atherosclerotic plaques	T	2.08		1.42		1.47		1.11		1.20		4.70	
	M	1.86		1.46		1.13		1.16		1.17		4.22	
	F	2.31		1.38		1.82		1.07		1.14		5.18	
Intimal necrotic areas	T	1.95		1.72		1.12		1.21		1.09		4.69	
	M	1.64		1.63		1.19		1.10		1.02		3.64	
	F	2.27		1.82		1.05		1.11		1.17		5.75	
Incorporated microthrombi	T	—		2.64			1.08	1.44		1.38		4.37	
	M		1.40	4.40			1.22	1.55		1.07		4.28	
	F	1.38		1.61		1.10		1.12		1.69		4.46	
Fatty streaks	T	2.66		1.11		1.06		1.05		1.01		3.90	
	M	2.66		1.15		1.08		1.05		1.00		3.88	
	F	2.67		1.08		1.05		1.05		1.02		3.92	

T, total; M, males; F, females.

From Velican, C. and Velican, D., *Atherosclerosis*, 47, 131, 1983. With permission.

Table 7

AGE-RELATED CHANGES IN THE PERCENTAGE OF SUBJECTS WITH ATHEROSCLEROTIC PLAQUES AND IN THE HISTOLOGICAL FEATURE OF THE PLAQUES

Plaque histology	Age groups (years)								
	6—10	11—15	16—20	21—25	26—30	31—35	36—40	41—45	46—50
Total plaques	2	4	12	28	42	58	72	84	96
Fibromuscular plaques	2	3	6	10	11	10	11	10	12
Mucoid plaques		1	5	10	7	6	4	2	2
Foam cell-rich plaques			1	8	16	6	3	0	0
Necrotic plaques					6	13	12	8	7
Fibronecrotic plaques						10	23	38	43
Fibrohyaline plaques					2	13	19	26	32

Table 8
AGE-RELATED RATE OF PROGRESSION OF VARIOUS TYPES OF ATHEROSCLEROTIC LESIONS IN SUBJECTS WITH (A) AND WITHOUT (WA) ATHEROGENIC DEVIATIONS FROM THE COMMON TYPE OF DISTRIBUTION OF THE CORONARY ARTERIES[a]

Types of atherosclerotic lesions		Age groups (years)					
		16—20	21—25	26—30	31—35	36—40	41—45
Atherosclerotic plaques	T	5.8	11.8	16.8	20.9	23.3	27.3
	A	7.0	14.1	20.0	22.9	24.0	27.9
	WA	4.6	9.5	13.7	18.9	22.7	26.7
Intimal necrotic areas	T	5.7	10.7	18.3	20.1	22.8	25.0
	A	7.1	11.9	20.1	21.5	23.8	26.8
	WA	4.3	9.6	16.5	19.0	21.8	23.2
Incorporated microthrombi	T	1.4	1.4	3.7	3.4	4.9	5.9
	A	1.4	1.5	4.0	3.5	5.8	5.9
	WA	1.4	1.3	3.4	3.3	4.0	5.8
Fatty streaks	T	5.0	13.3	14.9	16.0	16.8	17.0
	A	5.0	13.7	15.2	16.3	16.8	16.9
	WA	5.0	13.0	14.7	15.7	16.8	17.1

T, total.

[a] Results are % of tissue sections.

From Velican, C. and Velican, D., *Atherosclerosis*, 47, 131, 1983. With permission.

sites of the coronary arterial tree may demonstrate that whereas the general rate of progression of early lesions appeared to be age dependent, the acceleration of this rate and the occurrence of particular cycles of evolution toward advanced lesions seemed to be dependent on the action of risk factors. Many of the results furnished by this method cannot be obtained using in vivo coronary angiography of post-mortem routine gross inspection.

The visualization of similar topographic sites placed in sequence according to age, sex, and anatomical branching pattern also allowed us to confront data recorded grossly with those recorded by light microscopy. This confrontation showed the existence of an unexpected discrepancy, which appeared in:

1. The recorded character of lesions
2. The meaning of normal intima, fatty streak, and fibrous plaque
3. The age group in which the earliest atherosclerotic lesions were revealed
4. The number and topography of atherosclerotic plagues in a given arterial segment
5. The relationship between early and advanced atherosclerotic lesions (Table 9)[146]

If the macroscopic presence of normal intima, fatty streaks, and fibrous plaques in a well-delineated topographic site of the coronary arterial tree was further documented by histologic examination of this site, a different structural pattern than that recorded grossly was often revealed. After the age of 50, only one third or even one fourth or areas grossly classified as normal intima were lesion free on microscopic examination. Many other examples presented in the aforementioned study showed that macroscopic examination of the coronary intimal surface is not the best method for detecting an actual normal intima. The chance to classify grossly a pathologic intima as a normal intima increased progressively with age reaching a maximum in elderly people.[146]

We used grossly the term fatty streak to define any well-circumscribed, superficial ac-

Table 9
LIGHT MICROSCOPIC FEATURE AND ITS MACROSCOPIC COUNTERPART OF CORONARY ATHEROSCLEROTIC LESIONS

Light microscopy	Gross inspection
Early Lesions	
Fibromuscular plaques	Normal intima
Gelatinous lesions	Normal intima
Small intimal necrotic areas	Normal intima
Incorporated microthrombi	Normal intima
Intramural thrombi	Normal intima
Fatty streaks	Fatty streaks
Intermediate Lesions	
Mucoid plaques	Normal intima
Foam cell-rich plaques	Fatty streaks
Confluent, large, basal, intimal necrotic areas	Normal intima
Mature gelatinous lesions	Pearly white fibrous plaque
Mature fatty streak	Pearly white fibrous plaque with abundant lipid content or a fatty streak-like lesion
Intramural thrombi (without or with areas of fatty degeneration)	Pearly white fibrous plaque of variable lipid content
Advanced Lesions	
Fibrohyaline plaques	Raised pearly white fibrous plaque
Necrotic plaque	Normal intima or elevated lipid spots
Fibronecrotic plaques	Elevated, smoothly surfaced fibrous plaques of variable lipid content
Plaques with hemorrhage, ulceration, and/or superimposed thrombi	Complicated plaques

cumulation of lipid material appearing to the naked eye as streaks, dots, spots, patches, etc. and staining distinctly with sudan dyes. These are small or large, regular or irregular, limited or extensive, isolated or confluent, and flat or slightly elevated. The microscopic counterpart of grossly recorded fatty streaks may appear as:

1. Subendothelial collections of lipid-filled cells in frozen sections or of foam cells in paraffin-embedded material
2. Clusters of lipid-filled cells in a gelatinous lesion
3. Clusters of lipid-filled cells covering an intimal necrotic area
4. Clusters of lipid filled cells in the superficial area of a nonraised fibromuscular or fibronecrotic plaque
5. Clusters of lipid-filled cells in a foam cell-rich plaque
6. Clusters of lipid-filled cells covering a nonraised, developing fibronecrotic plaque
7. Fatty degeneration of the subendothelial region of an intramural thrombus

Whereas in adolescents the correspondence between the fatty streaks recorded grossly and on microscopic examination could be 100%, this correspondence decreased to 55% in mature adults, and to only 25% in elderly people. Consequently, with aging we were obliged to reclassify histologically many lesions grossly recorded as fatty streaks.

Macroscopically, we used the term fibrous plaque to designate firm, elevated intimal lesions, which in the fresh state are pale, gray, glistening and translucent, and uncovered

or partially covered by sudanophil material. The microscopic counterpart is also an atherosclerotic plaque occurring as fibronecrotic or fibrohyaline. Some plaques with superficial collections of lipid-filled cells and a similar pathologic feature may be grossly recorded as either fatty streak or fibrous plaque, according to their degree of elevation. Theoretically, a lesion which is not raised cannot be classified as fibrous plaque and a lesion which is raised cannot be classified as fatty streak. In fact, small fibrous plaques are not raised lesions and many advanced fatty streaks are raised. In our opinion, this is not the most important source of error, but the focal aspect attributed by the term "raised lesion" to a process which is diffuse and involves the entire length of the proximal and sometimes of the intermediate segments of the major coronary arteries in mature adults and the elderly.

This method of successive observations of similar topographic sites placed in sequence according to age also allowed us to detect important discrepancies between gross inspection and light microscopy concerning the onset of atherosclerotic lesions. In the left anterior descending artery, the earliest fibromuscular plaques were seen microscopically in children aged 6 to 10 years, whereas on gross inspection the first raised fibrous plaques were seen in young adults 21 to 25 years old. The earliest fatty streaks were recorded by both gross inspection and light microscopy within the second decade of life. This means that in the left anterior descending artery, the earliest fibromuscular plaques appeared microscopically a decade earlier than the first fatty streaks, whereas grossly the earliest fatty streaks occurred a decade earlier than the first raised fibrous plaques.

The earliest gelatinous lesions, intimal necrotic areas, incorporated thrombi, and intramural thrombi also appeared in our material before the onset of the first fatty streaks visible on gross inspection. On the other hand, isolated clusters of foam cells can be seen "by chance" in the coronary arteries of children starting from the postnatal period.

The method of macro- and microscopic observation of similar topographic sites of the coronary arterial tree placed in sequence according to age allowed us to demonstrate the existence of a *neglected coronary atherosclerosis*. The magnitude of this can be more clearly delineated if we refer to a study carried out in our laboratory on 800 apparently healthy subjects, aged 1 to 65 years who died accidentally (mainly in traffic accidents) and on 200 hospitalized patients aged 50 to 75 years who died of coronary heart disease. The coronary arteries and their main branches with an external diameter up to 1 mm were cross-sectioned serially at 2 to 3 mm and the degree of luminal narrowing was recorded in each sample, whereas the vessels with a diameter of less than 1 mm were examined only by light microscopy. The results showed that a neglected coronary atherosclerosis existed in all cases, being more clearly detectable in mature adults and the elderly.

Thus, for instance, of 100 subjects 51 to 55 years old, we found in 52 the presence of stenotic plaques in the longitudinally opened major coronary arteries (proximal segment). These lesions narrowed more than 50% of the arterial lumen of undistended vessels. If the proximal segment of the main branch vessels was also investigated, the number of subjects with more than 50% luminal narrowings increased in the same population sample from 52 to 61. Finally, if the results of light microscopic examination of the vessels with an external diameter of about 1 mm were added, the number of subjects with more than 50% luminal narrowing increased again in the same population sample from 61 to 73. To summarize, *in the same population sample*, including 100 mature adults 51 to 55 years old, 52, 61 or 73 of subjects exhibited atherosclerotic plaques encroaching upon more than 50% of the vessel lumen, *according to the method used*. Some illustrative results are present in Tables 10 to 12.

As already stated, it seems unrealistic to assume that all atherosclerotic lesions are visible grossly and after recording them, what remains is normal intima. Stenotic atherosclerotic plaques are often present in branch vessels which are routinely not opened by pathologists and not removed for light microscopic examination. There are also lesions related to ath-

Table 10
PERCENT OF CASES WITH CORONARY NARROWINGS (MORE THAN 50% IN UNDISTENDED VESSELS)[a]

Cause of death	Method 1	Method 2	Method 3
Sudden cardiac death	57	64	75
Acute myocardial infarction	61	74	87
Accidental death	38	47	58
Total	52	61	73

[a] Three methods were used on the same coronary arterial tree: (1) gross inspection of transacted proximal and intermediate segments of the major coronary arteries; (2) method 1 associated with gross inspection of the transacted proximal segment of the first diagonal, first septal, left marginal, right marginal, and posterior descending vessels; (3) the above-mentioned methods associated with light microscopy of the vessels supplying the conduction system and of other vessels approximately 1 mm in external diameter.

Table 11
NUMBER OF STENOTIC PLAQUES (MORE THAN 50% LUMINAL NARROWING) IN THE MAJOR CORONARY ARTERIES IN 95 SUBJECTS[a]

Vascular segment studied	Cause of death	No. of stenotic plaques: Method 1	Method 2	Method 3
Left main coronary artery	SCD	1	1	1
	MI	6	6	6
	AD	0	0	0
Proximal segment of the anterior descending artery	SCD	6	6	6
	MI	12	12	12
	AD	5	5	5
Intermediate segment of the anterior descending artery	SCD	2	2	4
	MI	3	3	4
	AD	1	1	2
Proximal segment of the left circumflex artery	SCD	4	4	4
	MI	10	10	10
	AD	2	2	2
Intermediate segment of the left circumflex artery	SCD	0	0	2
	MI	0	0	2
	AD	0	0	2
Proximal segment of the right coronary artery	SCD	3	3	3
	MI	5	5	5
	AD	1	1	1
Intermediate segment of the right coronary artery	SCD	5	5	5
	MI	12	12	12
	AD	1	1	1

[a] Subjects were 51—55 years old who died of sudden cardiac death (SCD), acute myocardial infarction (MI), and accidental death (AD). Methods 1, 2, and 3, see Table 10.

Table 12
NUMBER OF STENOTIC PLAQUES (MORE THAN 50% LUMINAL NARROWING) IN THE MAIN BRANCH VESSELS OF THE MAJOR CORONARY ARTERIES IN 95 SUBJECTS[a]

Vascular segment studied	Cause of death	No. of stenotic plaques		
		Method 1	Method 2	Method 3
First diagonal artery	SCD		2	3
	MI		5	6
	AD		2	2
First septal artery	SCD		2	3
	MI		2	5
	AD		2	1
Left marginal artery	SCD		1	1
	MI		1	1
	AD			1
Sinus node artery	SCD			3
	MI			2
	AD			1
Right marginal artery	SCD		1	2
	MI		1	2
	AD		1	1
Atrioventricular node artery	SCD			4
	MI			2
	AD			1
Posterior descending artery	SCD		3	3
	MI		5	6
	AD		1	2

[a] The subjects were 51 to 55 years old who died of sudden cardiac death (SCD), acute myocardial infarction (MI), and accidental death (AD). Methods 1, 2, and 3, see Table 10.

erosclerotic involvement which cannot be seen with the naked eye, but only on light microscopic examination, fibromuscular plaques, small gelatinous lesions and intimal necrotic areas, incorporated microthrombi, and intramural thrombi. Their light microscopic feature is illustrated in Figure 46. They are all considered early lesions, but can be detected during childhood, adolescence, and adulthood (even in subjects 50 years old), and represent an integral part of the neglected coronary atherosclerosis.

In our material, from 1000 subjects of all ages investigated, 420 (42%) exhibited gelatinous lesions and intimal necrotic areas and 190 (19%) intramural thrombi on light microscopic examination; all these lesions were missed by gross inspection. In the coronary arterial tree of these 100 subjects, there were 902 gelatinous lesions and intimal necrotic areas and in addition, 218 intramural thrombi in vascular segments recorded grossly as normal intima. The proportion of subjects with gelatinous lesions and intimal necrotic areas undetected by gross inspection augmented from 2.5% in children aged 6 to 10 years, to 14% in adolescents aged 16 to 20 years, to 53.8% in young adults 26 to 30 years old, and to 97% in mature adults 46 to 50 years old. Also, the proportion of subjects with intramural thrombi undetected by gross inspection increased from 1.5% in children 1 to 5 years old to 10.2% in adolescents 16 to 20 years old, to 14.8% in young adults aged 26 to 30 years, and to 33% in mature adults aged 46 to 50 years. This clearly indicates that the number of atherosclerotic lesions which remain undetected grossly increases progressively with age.

Of the 1000 subjects aged 1 to 75 years, 390 (39%) exhibited various histologic types of atherosclerotic plaques on light microscopic examination: fibromuscular, mucoid, foam cell-rich, necrotic, fibronecrotic, and fibrohyaline plaques. From the total of 1330 plaques revealed by light microscopy in the 1000 coronary artery trees examined, only 886 were also recorded grossly. This means that 443 or *33% of the existing plaques remained undetected* owing to the limits of the routine autopsy protocols. They represent one third of all atherosclerotic plaques developed in the coronary arterial tree of 1000 children, adolescents, young and mature adults, and elderly people up to 75 years old.

In our material, the ratio of atherosclerotic plaques detectable grossly to atherosclerotic plaques detectable microscopically was 0.63 in subjects 21 to 30 years old, 0.68 in subjects 31 to 40 years old, 0.76 in subjects 41 to 50 years old, and 0.78 in subjects 51 to 60 years old. This ratio shows that the number of plaques revealed by light microscopy remained constantly greater in successive age groups than the number recorded during gross examination. It demonstrates the existence in all age groups of grossly undetected plaques, even in subjects 51 to 60 years old.

It can safely be concluded that strict adherence to the routine autopsy protocols inevitably leads to the identification of only part of coronary atherosclerotic involvement, namely the lesions visible to the naked eye and present in the proximal segment of the major coronary arteries. This part might represent in some individuals 10%, in others 30, 50, or 70% of the real extension of lesions, the proportion of 70% being considered the upper limit recorded in our material. The pathologist must be aware that in each case submitted to post-mortem examination, an important number of atherosclerotic plaques remain undetected. If all these plaques would be without clinical significance the unrealistic aspects of routine autopsy protocols would appear to be of minor importance. Unfortunately, many grossly undetected occlusive lesions are present in sites and vessels involved in myocardial infarction or sudden cardiac death. A grossly undetected occlusive plaque in the vessels supplying the conduction system may be more significant for realistic clinico-pathologic correlations than some non-raised or raised plaques present in the proximal segment of the major coronary arteries. This assumption is also valid with respect to the presence of obstructive lesions in the intermediate and distal segments of the major coronary arteries, as well as in the proximal segment of the posterior descending, first diagonal, first septal, and marginal vessels.

II. EXPLORATIONS DURING LIFE: CORONARY ANGIOGRAPHY

A. Introductory Remarks

During the first half of this century, the extension and severity of coronary atherosclerosis was recorded by means of post-mortem observations only. In the second half, physicians and particularly cardiologists began to explore the extension and severity of coronary atherosclerotic involvement by means of a new technique applicable in the living patient: X-ray anatomy of the coronary arteries or selective coronary angiography.

The pioneers of this technique, such as Readner, Di Guglielmo, Arnuf, Paulin, and especially F. Mason Sones merit special attention. In the 1960s Sones and Shirley[147] and Judkins[148] reported effective means of visualizing the coronary arterial tree radiographically in patients with coronary heart disease. The technique combines selective cannulation of individual arteries, injection of contrast agent, and simultaneous cineangiography. We believe the name Sones will remain inseparably associated to the concept of selective coronary angiography.

Quantitative coronary angiography was also proposed a decade ago[149] and applied a few years later.[150] It requires high quality coronary arteriograms taken in two views at 90° to one another and seems to be of great value in determining progression or regression of coronary atherosclerotic involvement. Likewise, attempts are made to show, by quantitative

arteriography, how coronary flow reserve is related to, or might be predicted from, stenosis dimensions.[151]

Coronary angioscopy can be a useful adjunct to angiography by providing the added dimension of the cross-sectional view of obstruction, shape of the stenotic lumen, and general topography of lesion.[152] Both coronary angiography and angioscopy have their own unique advantages and limitations and each diagnostic technique can serve to complete the other.

Coronary arteriography is now not only an adequate method to detect coronary luminal narrowings in living subjects, but also a therapeutic tool thanks to Grüntzing, who developed coronary transluminal angioplasty and to Rentrop who performed coronary recanalization by fibrinolytic means (see Chapter 5).

Since its development in the early 1960s coronary angiography has occupied a position of foremost importance in the assessment of patients with coronary atherosclerosis and myocardial clinical manifestations induced by the obstructive character of atherosclerotic lesions. Angiographic pictures of the coronary arterial bed can provide a large amount of data not only on the obstructive character of certain atherosclerotic plaques, but also on the branching pattern of the coronary arteries, vessel diameters, branch angles, and many other anatomical particularities. Cinecoronary angiography is also able to detect cyclical rhythmic coronary obstructions produced by myocardial bridges with each cardiac systole.

It is likewise noteworthy that the wide use of coronary angiography and the possibility offered to quantitate coronary atherosclerotic involvement in the living subjects have attracted a new group of research in the field of atherosclerosis research: electronic engineers, bioengineers, computer scientists, physicists, biologists, mathematicians, etc.

Despite shortcomings in interpretation due to inter- and intraobserver variability and to selection biases (coronary angiograms are usually performed in a subset of patients with chest pain who are not a random sample of the active population), coronary angiography has become the most important technique in assessing in the living patients the presence of narrowings of possible clinical significance and the pathophysiologic effects of coronary stenoses. It allows us to correlate the development, extension, severity, and topography of atherosclerotic plaques with the myocardial clinical manifestations. "What has been learned about atherosclerosis by angiography can be compared to what has been learned about pulmonary tuberculosis by chest X-ray."[153] This particular value attributed to selective coronary angiography once again placed morphology in a prominent position.

Pathologic changes demonstrated by the injection of a contrast medium became the principal, and in many cases the sole, method able to reveal in the living patient the obstructive character of coronary atherosclerotic involvement. This is usually associated with data on coronary anatomy, cardiac function, collateral vessels, etc. The other invasive and noninvasive methods are far less important for the study of the natural history of coronary atherosclerosis than selective coronary angiography. Of particular importance for the cardiologist are quantitative analyses of lesional changes on serial arteriograms, as well as the association of coronary arteriography with exercise tolerance tests, injection of various radionuclides into the coronary arteries, administration of various pharmacological products, atrial stimulation with measurement of the products of cardiac metabolism in the coronary sinus, and other associated techniques. Many data are also offered by an adequate coronary angiography on the origin of the major coronary arteries, their epicardial course, collateral or terminal character of the main branch vessels, angle of branching, number of branch sites, presence, and size of collateral.[154]

Coronary angiography is not only used as a diagnostic and therapeutic tool, but when adequately performed, it may be employed to deduce the prognosis in patients with chest pain, abnormal electrocardiogram, arrhythmias, and other myocardial clinical manifestations. In particular, cinecoronary angiography may represent a valuable method for the long-term prognosis of symptomatic and asymptomatic patients with stenotic atherosclerotic plaques

in the coronary arteries. A normal coronary arteriogram should augur a good prognosis, especially when minor anomalies in the origin and distribution of the coronary arteries are also absent. A good prognosis is also reinforced by data obtained from coronary arteriography supplemented with those furnished by catheterization of the left atrium and ventricle, with recording of pressure changes and with those obtained from left ventriculography; all these data may provide a wealth of hemodynamic information needed to assess left ventricular function. This information is of particular value if a direct myocardial revascularization is necessary, the results of the surgical procedure being largely dependent on detailed knowledge of:

1. The anatomy of the coronary arterial tree
2. The localization, length, and severity of stenotic atherosclerotic plaques
3. The quality and size of the runoff
4. The viability of the myocardium beyond the obstruction and adequacy of the left ventricular function
5. The presence, number, and size of collateral vessels which provide alternate routes for delivery of blood to the myocardium distal to the stenotic or occlusive lesion

Coronary angiography is also the unique method which allows a clear diagnosis of left main coronary artery stenosis, since there are no historical or clinical signs which are specific for this particular localization of obstructive atherosclerotic plaques. As a rule, the progressive occlusion produced by such plaques is likely to be accompanied by severe chest pain and important ST depression with exercise in a manner undistinguishable from other patients with widespread atherosclerotic involvement of the coronary arterial bed. This is the reason the diagnosis of a stenotic lesion of potential clinical significance of the left main coronary artery ultimately depends on coronary angiography.

Coronary angiography, the most important method for the study of the natural history of coronary atherosclerosis in the living individual is, unfortunately, not free from complications. It requires hospitalization of the patient, is relatively expensive, and entails exposure to X-rays.

There is little controversy regarding the usefulness of coronary arteriography in separating cases without from those with severe stenotic atherosclerotic plaques.[155] On the other hand, the capacity of coronary arteriograms in predicting the hemodynamic significance of plaques which produce a mild to moderate degree of luminal encroachment still remains controversial.[156] It also should be pointed out that except for stenotic atherosclerotic plaques, all other types of atherosclerotic lesions go undetected.

In many subjects it is not possible to reveal consistent relations between myocardial clinical manifestations and the extent and severity of atherosclerotic involvement as seen on coronary arteriography. Severe stenotic lesions exist in apparently healthy subjects with a normal lifestyle and it is tempting to deduce that the respective lesions are bypassed by collateral vessels. In such cases, evaluation of the ischemic significance of luminal narrowing is a very difficult task. Conversely, cases with mild stenosis may show severe myocardial clinical manifestations associated with angiographic absence of collateral vessels. This points to the necessity of discussing the significance of the concept of "critical stenosis".

In many cases anginal pain, severe arrhythmias, or even myocardial infarction exist with no angiographic detectable lesions. An angiographer considers a coronary artery to be normal when smooth walls taper toward the periphery, all important branches are visible, and the luminal diameter is in agreement with age and sex. This normal coronary artery on an in vivo angiogram may show on post-mortem examination nonobstructive atherosclerotic plaques, very thick intimas, intramural thrombi, intimal necrotic areas, and many other types of lesions. Angiographers tend to disregard lesions which do not appear to affect the blood

flow, but some of these lesions include necrotic areas and may ulcerate, favor thrombus formation, and may produce peripheral embolization.

Dilation of the coronary arteries currently induced during coronary angiography by means of nitroglycerine not only provides better visualization of the stenotic atherosclerotic plaques and collateral vessels, but also eliminates the possibility of luminal narrowing due to a focal or segmental coronary vasospasm. On the other hand, in some patients with spontaneous angina pectoris, particularly the unstable form, coronary angiography is used to reveal the occurrence of spasm produced after the administration of ergonovine or related substances. The quality of the film and the images, the validity and precision of the method used to measure percent stenosis, the number of projection used, and the judicious administration of nitroglycerine to eliminate spasm may all contribute to the objective character of the results.

The interpretation of the same film by an angiographer can vary in two successive visualizations, particularly when they are separated by a long period of time. According to certain results, the rate of error may be as high as 20% and a 75% stenosis was evaluated incorrectly 31 times out of 100.[157] The rate of disagreement among four analysts in grading stenosis of the left anterior descending artery was as great as 50%.[158] False positive results are usually more numerous than false negative results and sometimes knowledge of the patient's history, particularly of the advanced clinical stages, may lead to overevaluation of the severity of the narrowing produced by atherosclerotic plaques. False results are sometimes obtained regarding the angiographic anatomy of the coronary arteries. When, for instance, injection of the contrast medium is not made in both right and left coronary arteries, it is possible to visualize an occlusion of the proximal segment of the left anterior descending artery and to overlook the fact that the patency of the distal segment of the respective vessel is maintained by collaterals originating from the conus artery that is inadequately opacified because of its separate ostium in the right sinus of Valsalva.[159] Lesions in distal segments of the major coronary arteries and in the proximal segment of the main branch vessels are difficult to judge due to decreased vessel size, increasing tortuosity, and failure of contrast material to fill these segments adequately. Error may occur not only because occlusive lesions are hidden by vessel overlap, but also because arterial spasm may be interpreted as a fixed obstruction. While reports of errors in coronary angiography diagnosis can often be found in the available literature, they are not more important than those found in other areas of medical diagnosis where human judgment must ultimately be translated into quantitative terms.

Angiographers consider that atherosclerotic lesions which produce lumen obstruction are only focal in nature. They record a focal disease separated by apparently uninvolved angiographic vascular areas. In fact, particularly in mature adults and especially in patients with coronary heart disease who require angiographic examination, the focal stenotic or occlusive plaques are superimposed over a diffuse atherosclerotic involvement of the whole proximal segment of the left anterior descending and left circumflex arteries and the proximal and intermediate segments of the right coronary artery. As has been repeatedly mentioned, histopathologic techniques used to quantitate raised atherosclerotic lesions pose similar problems, since raised focal fibrous plaques are superimposed over a diffuse atherosclerotic involvement that is erroneously recorded as normal intima.

The existence of this diffuse atherosclerotic involvement inevitably leads to underestimation of the lesion severity. Better analytical methods to reveal angiographically this usually overlooked diffuse atherosclerotic involvement are urgently needed. In addition to the universal use of relative percent diameter narrowing as a clinical measure of severity of atherosclerotic involvement, other geometric characteristics of the diseased coronary artery wall would be taken into consideration, such as succession of multiple lesions in series, length of atherosclerotic involvement in each vascular segment, absolute diameter of lesions of

possible clinical significance, prevalence of eccentric or concentric narrowings, and kinking and scalloping of a coronary artery border and many other parameters.

B. The Concept of Critical Stenosis

The general belief that not all stenoses in the coronary arterial tree need to be hemodynamically significant and consequently play a potential role in myocardial clinical manifestations, has led to the concept of "critical stenosis". This concept relates lesion anatomy and severity to its functional consequences and especially to changes in coronary flow reserve. In normal coronary arterial trees a well-defined ratio of maximum coronary flow after a significant vasodilatory stimulus to resting flow can be recorded. This capacity to vasodilation which allows a three- to fourfold increase in coronary flow reserve becomes altered in the presence of coronary stenosis associated with the development of an inadequate collateral circulation. When this collateral circulation is absent, coronary flow reserve limitation may be considered proportional to the severity of coronary stenosis. Moreover, coronary flow reserve may become impaired even with mild stenoses narrowing 30 to 40% of the vascular lumen if the lesion is 3 to 12 mm long.[151] Stenoses produce a progressive passage from a low-flow, high-resistance coronary circulation to a high-flow, low-resistance circulation. Moreover, as the stenosis becomes sufficiently severe to create a resistance comparable to that of the distal vascular bed, the distal bed vasodilates and loses its ability to autoregulate.[151]

Many investigators accept the view that the degree of coronary luminal narrowing, detected angiographically, at which the resistance to flow becomes able to impair myocardial oxygen supply can be defined as critical.

From the point of view of the angiographer, a critical stenosis may be a lesion which produced more than 75% luminal insufficiency, irrespective of its hemodynamic consequences and the availability of the collateral circulation. The cardiologist considers that a critical stenosis is a lesion which determines symptoms of myocardial ischemia at rest or other types of myocardial clinical manifestations. There are also different interpretations of the term "critical stenosis" given by pathologists, pathophysiologists, experimentalists, etc.

Some investigators define critical stenosis in terms of the lesion area as measured in cross-sections, others as decrease in vessel diameter. Certain results show that a luminal insufficiency of 80% was necessary to reduce myocardial blood flow significantly when the stenosis was brief and 60% when the stenosis was extended.[160] This significant reduction in myocardial blood flow means that only the resting coronary flow may remain available to the contracting myocardium and this may be reflected in the occurrence of signs of myocardial ischemia during exercise. In the great majority of studies such clinical and/or electrocardiographic signs were detected when the atherosclerotic plaques narrow up to 75 to 80% of the coronary artery lumen and an inadequate collateral circulation exists. If a circular narrowing is involved, a 75% decrease in area corresponds to a 50% decrease in diameter and some investigators consider significant luminal insufficiency when a stenotic lesion narrowing by more than 50% the original lumen is present in at least one major coronary artery.[161]

All attempts to define and delineate what a critical stenosis may be must refer only to lesions which are not bypassed by collateral vessels and this is sometimes an insurmountable difficulty both in man and in laboratory animals. Figure 49 is very demonstrative in this respect. Unfortunately, many clinicians, and especially many cardiologists, overlook the existence of collateral vessels and accept the existence of "magic" percentages of luminal narrowing, starting from the unrealistic assumption that coronary arteries are end arteries.

An usually neglected factor is also the length of the stenosis. In the presence of a similar degree of luminal narrowing of 75%, the stenosis of greater length will restrict myocardial flow more than the shorter one. Moreover, the existence of moderate stenoses may provide additive effects like resistors in series, further restricting coronary flow. Of interest is the presence or absence of poststenotic dilation.

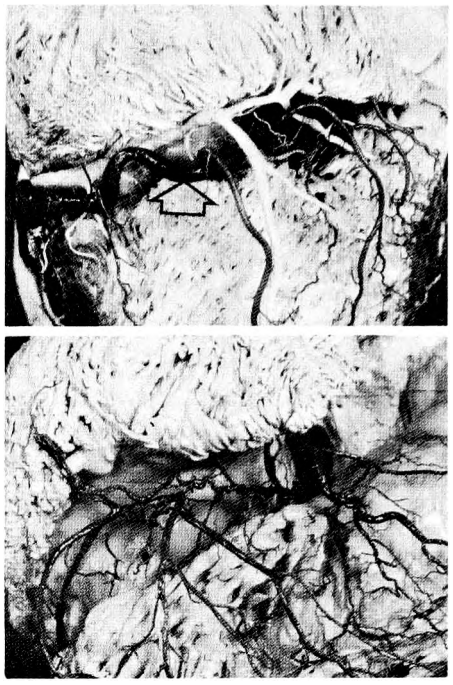


FIGURE 49. Normal right coronary artery (arrow, top) and the same perspective as an occluded right coronary artery (bottom). Expanded vasa varosum from corkscrew connection between proximal and distal segments of occluded artery. (From James, T. N., *Circulation*, 47, 189, 1970. With permission.)

When a circumflex artery is hypoplastic and short, ending as the left marginal branch, the significance of a critical stenosis in its proximal segment is different than when a left circumflex artery is well developed, gives rise to vessels to the conduction system, and supplies the crux cordis. Many angiographers and cardiologists overlook these elementary anatomical aspects and tend to correlate quantitative measurements of coronary luminal narrowing only with anginal pain and other clinical parameters.

A luminal narrowing which reduces 75% of the original lumen may have dramatic consequences in the left main coronary artery, but is not significant in a right coronary artery which does not give rise to the vessels supplying the conduction system. On the contrary, a 75% stenosis of the right coronary artery at the branching point of the atrioventricular node vessel may be associated with severe arrhythmias and even with sudden cardiac death. A similar 75% stenosis in the left anterior descending artery may give rise to small areas of myocardial necrosis, whereas in the proximal segment of the first diagonal or first septal branches, it may produce myocardial infarction in the anterior wall of the left ventricle or in the anterior interventricular septum.

We have often observed that the same critical stenosis may produce in some cases small areas of myocardial ischemia, in other cases typical myocardial necrosis in the subendocardial region, and in still others the evolution from a preexisting subendocardial infarction to a transmural one.

A similar 75% critical stenosis has different hemodynamic consequences in the left and right coronary arteries, since the left main coronary artery carries five times more blood to the left ventricle than the right coronary artery.

A critical stenosis may also be influenced by some dynamic changes which occur in the lesion itself and reduce or increase its resistance to flow.

The tendencies to oversimplification and to consider all luminal narrowings of 75% as of equal functional and clinical consequences are unrealistic. Factors that determine coronary flow limitation include, in addition to the degree of luminal narrowing: length of the stenotic lesions and many other geometric factors, blood viscosity, arterial pressure, development of collateral vessels, the capacity of small coronary vessels to autoregulate, nervous influences, etc. The real value of coronary critical stenoses may also be largely influenced by the requirement of the mass of viable myocardium beyond the stenosis.

Since arteries with different flow loads and different areas of supply have different critical stenosis values, a similar degree of luminal narrowing (1) may be incompatible with life in the left main coronary artery, (2) may cause unstable angina when present in the proximal segment of the left anterior descending artery, (3) may produce severe arrhythmias when present in the right coronary artery, (4) may give rise to total bundle branch block when present in the first septal artery, and (5) may induce posterior myocardial infarction when present in the posterior descending artery, anterior myocardial infarction when present in the first diagonal artery, and small areas of myocardial necrosis when present in the left or right marginal arteries. This variability of consequences related to the existence of critical stenoses in the various segments of the coronary arterial tree demonstrates the necessity for a case-by-case deep analysis of the aortic origin, epicardial course, caliber, type of branching, number and diameter of the main branch vessels, collateral or terminal character of these branch vessels, angle of branching, presence, and size of collaterals. Strongly related to this important facet of coronary angiography, it may be of interest to mention here this confession: "It is discouraging to me to see how many young investigators are keenly interested in learning how to perform coronary arteriograms, but have not made even rudimentary attempts to learn the anatomic distribution of the coronary arteries."[163]

Coronary arteriography does not define the physiologic consequences of various degrees of luminal narrowing produced by atherosclerotic plaques and the most eloquent example is the frequently used term "one vessel disease". It refers to a major coronary artery with a critical stenosis, usually more than 75% luminal narrowing, whereas the other major coronary arteries may have stenotic lesions encroaching 40, 50, or 60% of the vessel lumen. This means that *a diffuse atherosclerotic involvement may be presented as one vessel disease* only if a sole stenotic lesion is considered to show a critical stage of lumen encroachement. According to this concept of a single vessel disease, a patient with 100% obstruction of the proximal segment of the left anterior descending artery may have the same atherosclerotic involvement features as a patient with 75% obstruction in a similar vessel. Both cases are grouped together as having one vessel disease when the natural history of coronary atherosclerosis is evaluated.

The magnitude of relative flow reduction necessary to cause anginal pain, collateral development, and myocardial necrosis is not well defined since the physiologic effects of critical stenosis cannot be accurately determined by conventional angiographic approaches.[164] In fact, even the degree of coronary narrowing is not accurately evaluated by angiography, as the data recorded can be strongly influenced by the existence of multiple narrowings and the length of each stenotic lesion. A series of narrowings create multiple sites of wave reflection, turbulent flow, and other hemodynamic changes which are far more complex and more atherogenic than those produced by a single isolated stenotic lesion. It is very difficult to deduce these complex interactions from a simple quantification of the degree of luminal narrowing as it occurs when determined by high-quality coronary arteriograms.

Many investigators considered that there are not, in fact, identifiable characteristics of critical coronary stenosis. The prevailing custom of expressing arteriographic images of stenotic atherosclerotic plaques in terms of percent luminal narrowing implies a precision in measurement which does not exist. On the other hand, clinical decisions are often based on these numerical presentations of coronary arterial stenosis accepted as accurate. This

accuracy would require the knowledge of exactly what the hemodynamic consequences of a critical stenosis are and to consider coronary circulation as including stenotic lesions which are not bypassed by collateral vessels. This cannot be accepted, since patients with total obstruction of a major coronary artery (except the left main coronary artery) may have sufficient collateral development in order to preserve an asymptomatic state and an adequate left ventricular function. In addition, in many studies the degree of coronary luminal narrowing appeared unrelated to altered ventricular function.[165] Patients with variant angina may have only "minimal" coronary atherosclerosis; on the other hand, moderate angina pectoris is often associated with severe multivessel involvement. In essence, the existence of multiple stenoses, the variability of their geometric features, the presence or absence of collateral vessels, the preexistence of myocardial necroses, and of compensatory myocardial hypertrophy may all dramatically alter the relationship between a critical stenosis and myocardial clinical manifestations.[166]

C. Angiographic-Pathologic Correlations

In most coronary arteriography laboratories grading is semiquantitative, with stenoses classified in stages. The following scale has been proposed by the American Heart Association AD Hoc Committee:[167]

— normal (absence of narrowing)
25% stenosis (reduction in luminal diameter not exceeding 25%)
50% stenosis (reduction in luminal diameter of 26 to 50%)
75% stenosis (reduction in luminal diameter of 51 to 75%)
90% stenosis (reduction in luminal diameter of 76 to 90%)
99% stenosis (lumen as fine as a hair or subtotal occlusion)
100% stenosis (total occlusion)

Certain angiographers group together all stenoses below 50% or between 75 and 95%.

The first attempts to correlate the degree of luminal narrowing to myocardial clinical manifestations showed that there is no pattern of coronary artery involvement pathognomonic of a specific clinical syndrome included in the large sphere of coronary heart disease.[168] In chronic, stable angina, involvement of one, two, or three vessels may show virtually equal frequency, for instance, 31, 37, and 32%, respectively.[169]

In a group of 1550 patients suspected of having coronary heart disease and explored consecutively, the frequency of stenotic atherosclerotic plaques was 83.7%, but only 73.6% showed a reduction of more than 75% in the diameter of the major coronary arteries.[170] Among the above-mentioned 1550 patients suspected of having coronary heart disease, triple vessel involvement was detected in 40%, a double vessel involvement in 30%, and a single vessel involvement in 30% (16% in the left anterior descending artery, 5% in the left circumflex artery, and 9% in the right coronary artery).[170]

If such results are compared with those obtained on post-mortem investigations, many discrepancies may occur. First of all, the angiographer examines a distended vessel, the pathologist and undistended one. Second, the time interval between angiographic and post-mortem examinations may include months or years, and during this period important additional changes may develop which were not observed angiographically. Third, angiographic examination frequently detects stenotic plaques in coronary vessels that are neglected during routine autopsy, such as posterior descending, first diagonal, first septal, left marginal, and right marginal branches. A preliminary study of our laboratory demonstrated that one out of every three subjects with atherosclerotic plaques in the major coronary arteries also have atherosclerotic plaques in the proximal segment of the main branch vessels.[64] The presentation of the atherosclerotic involvement of the coronary arteries of mature adults limited to only

the major coronary arteries and particularly to the proximal segment of the major coronary arteries as is usually mentioned in autopsy protocols may constitute an important source of discrepancies between data furnished by gross inspection and in vivo coronary angiography. The existence of these discrepancies was frequency mentioned in the available literature.[171-182] An excellent correlation between luminal size, as determined angiographically and morphologically at the site of focal obstruction has been found only in an experimental porcine coronary atherosclerosis.[183]

More than two decades ago it was clearly demonstrated that in human coronary arteries many affected segments escaped radiologic detection, half of the segments that appeared normal or minimally involved angiographically showing severe atherosclerotic involvement at autopsy.[172] When pathologic examination revealed no narrowing, roentgenologic findings were similar; on the other hand, a normal coronary angiogram does not necessarily exclude the presence of atherosclerotic lesions and severe stenotic plaques were recorded in some cases with normal roentgenograms.

Likewise, more than 2 decades ago, a comparative study on the severity of luminal reduction graded by coronary arteriography and by pathologic examination showed a discrepancy in about 90% of the segments investigated. The severity of lesions was underestimated by arteriography in 86% and overestimated in only 3%.[173] When an overestimation of lesion severity is angiographically recorded, one is tempted to suspect that this may be due to inadequate filling of the vessel with contrast medium, abnormalities in coronary runoff, platelet aggregation, diffuse coronary artery vasoconstriction, or vasospasm.

The highest discrepancy rates in angiographic-pathologic correlation seems to be furnished by atherosclerotic involvement of the left main coronary artery. This vessel is usually well-opacified and visualized, without branching points and overlapping arteries. In spite of these optimal conditions, the antemortem angiographic investigations underestimate the post-mortem pathologic findings. Particularly moderate stenoses are associated with important discrepancies in interpretation and these discrepancies seem to be accentuated in the presence of ostial lesions and calcium deposits.[182] The accuracy of angiographic assessment of the left main coronary artery is very important since severe stenosis of this vessel may be associated with a high mortality which increases with augmented severity of luminal narrowing.

Likewise, the intermediate segment of the right coronary artery between the origin of the right marginal branch and posterior descending branch, is a vessel segment in which a poor correlation occurs between in vivo angiography and post-mortem gross inspection. On coronary arteriograms the proximal segment of the right coronary artery appears usually more severely involved by atherosclerotic plaques than the intermediate segment, whereas pathologic examination may show a more severe involvement of the intermediate segment.

Discrepancies between antemortem angiographic and post-mortem pathologic findings were frequently recorded at the level of branch ostia of the first and second diagonal vessels and of the main septal vessel, which are branches of the left anterior descending artery. The changes present at these branch sites are of particular value in assessing the mass of myocardium at risk and an adequate blood supply of the conduction tissue. Studies based on a computer multisegmental model of the coronary arterial tree revealed that stenotic lesions mainly develop adjacent to or proximal to the point of branching.[44] In the left anterior descending artery, the lesions were chiefly prevalent immediately after the first diagonal branch and at the origin of this branch, as well as of the main septal vessel. Failure to angiographically observe this concentration of stenotic lesions at branch sites appears to be due to the overlapping of radiographic images of the proximal segment of the first diagonal and septal vessels near their point of origin. Unless the site of a branch is perpendicular to the angle of viewing, such overlap may occur.[179] An aberrant or anomalous vessel superposed upon a normal vessel may create the angiographic supposition of the existence of a critical stenosis, but this supposition may be rejected when various projections are used. On the

other hand, failure to separate two superimposed vessels on the film may obliterate a critical stenosis.

The fact that the coronary artery lumen may appear normal or nearly normal in one projection of the coronary arteriogram, but in projections at right angles to this view may occur greatly narrowed, is a source of discrepancies in angiographic-pathologic correlations. The angiographer seems to be accustomed to visualize critical stenosis in some projections but not in others. A stenotic lesion may be seen in the segment parallel to the film in its true linear projection, whereas in perpendicular projection it may not be visible at all. Coronary arteriography requires various projections and multiple injections of the contrast medium to visualize stenotic lesions adequately, whereas the pathologist can detect such lesions very rapidly in longitudinally opened or cross-sectioned vessels.

In essence, if angiographic and pathologic findings are compared, it is possible to reveal:

1. Lesions considered as obstructive both angiographically and pathologically
2. Lesions interpreted as not obstructive both angiographically and pathologically
3. Lesions which appear as significant angiographically and insignificant pathologically
4. Lesions interpreted as insignificant or normal angiographically but significant pathologically as concerns their stenotic character

Since coronary angiography in life provides a view of the linear dimensions of the coronary artery lumen seen in its longitudinal view, and histologic examination provides a view of the cross-sectional area of the coronary artery, for any degree of longitudinal narrowing there is a proportional greater loss of the cross-sectional area.[181] A 50% reduction in lumen diameter may be associated with a 75% reduction in cross-sectional area. If a significant angiographic lesion is defined in the presence of a 50% luminal narrowing, an equivalent significant pathologic lesion must be accepted as a 75% reduction in the cross-sectional area of the coronary segment investigated.

Differences in the geometry of lesions may also have important effects on arteriographic estimates and on angiographic-pathologic correlations. The atherosclerotic plaques may appear as *concentric* or *eccentric* on coronary angiography. The eccentricity and significant deformity of arterial lumen may cause difficulties in arteriographic measurements of coronary stenosis, difficulties which require utilization of different projection angles.[184]

An important source of discrepancies between angiographic and pathologic findings is furnished by the irregularly shaped plaques. When the shape of the lesion is other than circular, this may produce overestimation or underestimation of the severity of luminal narrowing.[185] In unselected cases, a slit-like lumen was revealed in 29%, an eccentric polymorphous lumen in 40%, and a central lumen in only 31% of the coronary arteries.[186] The most difficult to interpret angiographically is the slit-like lumen since it may give rise to an image similar to that of a normal artery. This so-called slit-like lumen of angiographers is rarely seen if the coronary arteries are further studied at autopsy after in vivo coronary arteriography. An adequate explanation could be that the slit disappears when the portion of the vessel noninvolved by lesions collapses against the more rigid eccentric plaque. When the slit-like lumen persists in undistended vessels it poses no problems in estimating the degree of narrowing at necropsy. On the other hand, it continues to be a source of angiographic error when it allows the entire diameter of the artery to be opacified with contrast materials. Its pathologic significance may not be fully estimated if the short diameter of the lumen is not brought perpendicular to the X-ray beam by an adequate number of projections.[181]

An eccentric plaque is usually associated with an eccentric lumen which may appear either slit-like or polymorphous in shape, sometimes stellate. The slit-like lumen produced by an eccentric plaque has its greatest width in some projections as wide as the original lumen. As already mentioned, such narrowing lesions may be overlooked in arteriograms in spite of the fact that they may represent critical stenoses.

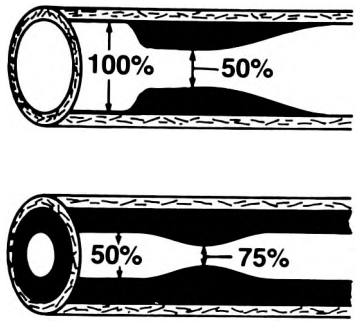


FIGURE 50. Diagrammatic representation of an angiographic view of two coronary arteries which have luminal plaques. Because the angiogram is a luminogram, and the width of the original arterial lumen is unknown, the least-narrowed segment is often presumed to be normal. Comparison of a narrowed segment to a near-normal segment (top) is more likely to occur in younger patients, whereas comparison of a severe narrowing to a less severe narrowing, which may be considered "normal" angiographically (bottom), is more likely in older patients. This situation leads to an underestimation of the degree of stenosis in older patients. (From Warnes, C. A. and Roberts, W. C., *Am. Heart J.*, 108, 431, 1984. With permission.)

In the great majority of studies the degree of luminal narrowing is estimated visually, using as a criterion the percentage reduction in diameter of the narrowed segment of the coronary artery, as compared with the diameter of the area proximal to the narrowing. This method of calculating the diameter of a narrowed coronary area by comparison with the diameter of the adjoining area may not be accurate if the portion of the vessel proximal of distal to the stenotic lesion has a thickened intima or a diffuse atherosclerotic involvement. Whereas the pathologist compares the residual lumen with the presumed original lumen, the angiographer compares the narrowed segment with the adjacent segment which may be not normal in mature adults and particularly in patients with coronary heart disease. Consequently, the angiographer frequently compares focal stenotic atherosclerotic plaques with adjacent diffuse atherosclerotic involvement (Figure 50). The absence of a normal arterial wall adjacent to a stenotic atherosclerotic plaque is an important source of errors, since the true baseline for which the arteriographer must estimate stenosis is unknown. Whereas the pathologist can record a presumed normal lumen starting from an area encircled by the internal elastic membrane the angiographer is obliged to compare, particularly in patients with coronary heart disease, a focal stenotic lesion with an adjacent thickened intima or diffuse atherosclerotic involvement.

If this adjacent segment is uniformly narrowed by 50% by a very thick intima and if the stenotic plaque reduces the coronary luminal diameter also by 50%, no area of stenosis will be identified angiographically. This is the reason the eminent pathologist William C. Roberts assumes, based on the results of his analytical studies, that "histological stenosis is not comparable with angiographic stenosis".[171]

We have repeatedly mentioned in this book that both angiographers and pathologists have many and sometimes insurmountable difficulties in order to evaluate the pathophysiologic significance of a stenotic lesion present in the coronary arteries in the absence of adequate data on the collateral circulation. More than four decades ago, it was observed that complete occlusions were found in the coronary arteries of 38 out of 355 autopsies, the respective cases being asymptomatic during life.[187] Subsequent studies also showed total occlusions, critical stenoses, or over 50% narrowing of one or more major coronary arteries without signs of myocardial ischemia.[188] Many investigators accept the logical view that the ischemic

significance of a coronary stenosis cannot be adequately evaluated in the absence of data on the compensatory role played by collateral vessels. All perfusion deficits are significant only if a stenotic plaque is not bypassed by a compensatory collateral circulation. The existence of "magic" percentages of luminal narrowing which could indicate the onset of myocardial ischemia cannot be accepted as long as we consider that coronary arteries are not end vessels. Unfortunately, the angiographer can detect only some large collateral vessels located on the surface of the myocardium and this may represent a small part of the collateral circulation available. Moreover, when collateral vessels have a diameter less than 0.1 mm they are overlooked. As concerns the pathologists, their information on collateral vessels is based only on data offered by angiographers.

An additional important problem related to angiographic-pathologic correlations refers to the so-called "complicated lesions" and particularly thrombosed plaques. These complicated plaques are easily identified by pathologists but represent a still-unsolved problem for many angiographers. Complicated atherosclerotic plaques show irregular borders and intraluminal lucency at the site of stenosis on the angiogram. Such changes may be seen on post-mortem angiograms and may not be apparent on a coronary angiogram of a living patient. This increases the likelihood of false negative results in attempting to diagnose complicated plaques by in vivo coronary angiography. A smooth narrowing without intraluminal lucencies is considered by certain angiographers a noncomplicated atherosclerotic plaque. On the other hand, a stenotic plaque with irregular borders or intraluminal lucencies would indicate a ruptured plaque, intraplaque hemorrhage, and an occluding or recanalized thrombus.[189]

An improvement in angiographic-pathologic correlations could also result from the attempts to quantify atherosclerosis in living subjects using angiographic and ultrasound techniques. A tendency also exists to replace visual estimation by computerized technique. This technical progress is indeed impressive, but it cannot offer adequate information on the preexisting state of the coronary arterial wall, the severity and extension of atherosclerotic involvement, the size and microarchitecture of preexisting and developing lesions, and the existence of lesions which may ulcerate and produce peripheral embolization.

D. Repeated Coronary Angiography

1. General Observations

The clinical course and prognosis of coronary heart disease are usually related to the severity of coronary atherosclerosis as assessed angiographically.[190-205] Moreover, successive angiographic examinations of the coronary arterial tree are possible and this represents the best-available method for investigating the progression or regression of coronary atherosclerotic lesions in the living subject and correlation of this evaluation with myocardial clinical manifestations. Usually, repeated angiography is performed only in patients who show clinical signs of progression of the stenotic lesions and therefore, the natural history of coronary atherosclerosis deduced from these studies is likely to be estimated as worse than it actually is. An ideal angiographic study of atherosclerotic plaque progression would include the initial angiographic evaluation of a group of asymptomatic subjects with apparently normal coronary arteries or with mild atherosclerotic involvement, followed by selective recatheterization after a predetermined interval of time. Sequential coronary arteriograms from children to mature adults would clarify many aspects concerning the natural history of coronary atherosclerosis, but such studies cannot be justified. Repeated arteriographic visualization of the coronary arteries is usually done only in patients with myocardial clinical manifestations. These repeated angiographic examinations are performed only when some changes in the patient's clinical state suggest that stenotic atherosclerotic plaques encroach significantly upon the coronary artery lumen or that unexpected complications have occurred. This restricts the area of retrospective studies to selected patients who show an unfavorable course of the natural history of atherosclerosis.

In spite of its limited use, repeated coronary angiography is the unique method which can assess *the evolution of the same coronary atherosclerotic plaque in a living subject.* It helps to determine the site and severity of stenotic lesions, its time-related change, and in addition, provides information about collateral vessels and distal runoff. The great majority of investigators in the field of coronary angiography believe that time is the major determinant of progression of stenotic atherosclerotic plaques toward lesions of possible clinical significance. Many investigators assume that the progressive luminal narrowing of a coronary artery, as revealed by repeated arteriograms, seems to occur in relation to time. All attempts to define a rate of progression of stenotic coronary atherosclerotic plaques appeared as very difficult tasks, sudden, unpredicted events intermittently punctuating the time-dependent natural history of coronary atherosclerosis.

Since certain atherosclerotic plaques may progress from nonobstructive to obstructive lesions, many clinical and surgical decisions are based on the premise that a partially obstructing plaque will, in time, completely occlude the coronary artery. This view oversimplified the natural history of coronary atherosclerosis because there are different types of plaques with different rates of progression: very low, low mild, fast, and very fast. The size of the vessel also plays an important role in this passage from a partially to a totally obstructive lesion. Moreover, some events may occur independently of time as a function of other variables. Total occlusion of a coronary artery, as revealed in coronary angiograms, is not necessarily caused by gradual growth of the plaque, but may be produced by other mechanisms, individually or in combination. Among these mechanisms rupture of the plaque, thrombus formation, and vasospasm seem to be the most important. It might be assumed that two processes, one time-dependent and one time-independent, determine the natural history of coronary atherosclerosis.[203]

It would be of particular importance to differentiate at the coronary angiographic examination progressive from nonprogressive atherosclerotic plaques, or slowly progressing lesions from rapidly progressing ones. The prevalence of nonprogressive atherosclerotic plaques might predict a stabilized period in the natural history of coronary atherosclerosis over years and decades; on the contrary, the prevalence of progressing plaques would lead to myocardial clinical manifestations.

When the rate of progression of coronary atherosclerotic plaques is slow, collateral vessels may develop in adequate number and size and may partly or entirely compensate the effects of stenoses; when this rate of progression is rapid, this usually leads to an inadequate collateral development.

Progression was more clearly detected in eccentric than in concentric stenoses.[205] The stenoses on which an occlusive thrombus developed were likewise eccentric or predominantly eccentric. All these observations suggested that the morphologic type and geometry of the lesion are important prognostic parameters.

In segments without visible coronary atherosclerotic plaques at the first angiographic examination, the frequency of progression at second examination is very low or low. In segments with atherosclerotic plaques showing a low or moderate degree of severity, progression seems to occur in proportion to the initial severity of atherosclerotic involvement and the interval between repeated angiographies. However, this progression does not continue indefinitely, since not all plaques become occlusive lesions. As the severity of stenosis produced by an atherosclerotic plaque will increase in time, the probability of plaque ulceration, rupture, hemorrhage, etc. will also be augmented, together with the probability of thrombus formation and of spasm occurrence. These latter occlusive events are not directly dependent on time, nor on the cumulative severity exhibited by the atherosclerotic plaque, but may be related to other unpredictable factors.

If a normal arterial segment at a first angiographic examination becomes totally occluded after 2 years at a second angiographic examination, this would indicate for an angiographer

a progression of the disease from 0 to 100%. If an arterial segment with an atherosclerotic plaque encroaching upon 70% of the arterial lumen in a first arteriogram had become totally occluded after 2 years in a second arteriogram, this would indicate a progression of the disease from 70 to 100%. The angiographer is not able to detect if the above-mentioned progression resulted from an intrinsic increase in plaque thickness, from continuous incorporation of microthrombi or the sudden development of an occlusive thrombus, or from a combination of these mechanisms.

The fact that not all plaques continue to progress is an intriguing problem for the angiographists, since additional tests are necessary to detect the initial severity of a stenotic lesion and its potential to become an occlusive one. This tendency of lesions to progress was revealed angiographically from 20% to more than 50% of cases.[206,207]

There are two sites of the coronary arterial tree where the opportunity to detect plaque progression is greater than in other neighboring regions: (1) the proximal segment of the left anterior descending artery near the branching sites of the first diagonal and septal vessels and (2) the intermediate segment of the right coronary artery proximal to the branching site of the posterior descending vessel. On the other hand, progression can rarely be seen in the left main coronary artery (except in its bifurcation area) and in the first centimeter distal to the aortic origin of the right coronary artery. According to some points of view, to assume that a progression exists would require the consensus of three observers on a 30% or greater decrease in luminal diameter.[208]

On a group basis, the major factors of prognosis seem to be time and severity of initial lesions. In individual cases, the rate of progression may appear independent of the initial severity of lesions, and also independent of topographic sites and presence of risk factors for coronary heart disease. This would indicate that no transfer of a statistical tendency from a group to a particular case is warranted. Additional parameters difficult to evaluate are the subjective selection of patients, differing indications for angiographic reexaminations, and greatly varying intervals between coronary angiograms. In essence, repeated coronary angiography is a very useful method to investigate the natural history of coronary atherosclerosis. On the other hand, this natural history includes a broad spectrum of pathological changes which are only partly identified by means of repeated coronary angiography.

2. Selected Examples

These are presented in a chronological order.

- Of 46 patients with apparently normal coronary arteries of minimal, nonsignificant atherosclerotic involvement, 44 did not manifest evidence of progression of lesions 52 months after an initial arteriogram.[170]
- The 5-year mortality in patients with single vessel involvement was 8.8% and in those with multiple vessel disease the mortality rate was 37%.[209]
- Sequential coronary arteriograms in patients with severe obstructive coronary atherosclerosis have shown that progression of lesions can be frequent, rapid, and highly predictable (Figure 51). It was observed to occur within 1 to 2 years in 52 to 76% of patients studied. After an average interval of 26 months, 33% of coronary obstructions worsened and coronary atherosclerosis progressed on an average of two arterial sites per patient. Subsequent progression was independent of overall severity of initial disease or prior disease at the site of progression and occurred frequently in previously apparently normal coronary arteries.[197]
- Between September 1966 and September 1976, a group of 48 patients with normal coronary arteries or nonsignificant atherosclerotic involvement documented in a first coronary arteriogram underwent a second arteriogram because of persistent recurrent chest pain. The interval between examinations was 13 to 108 months (mean of 42 months). The results suggested that lesions of possible clinical significance very rarely develop in previously normal coronary arteries and that roughly 75% of adults with normal coronary arteries or

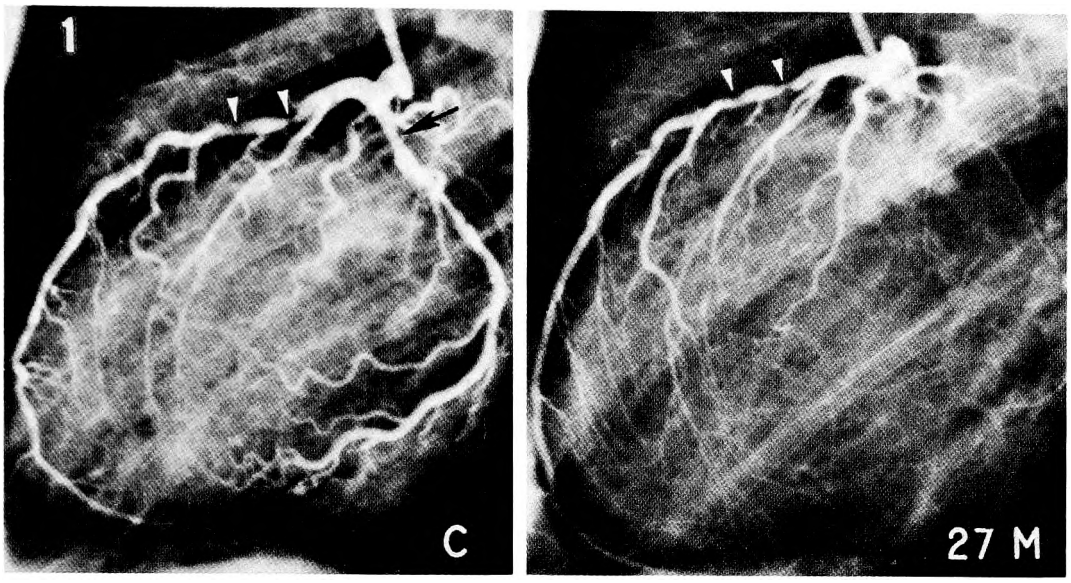


FIGURE 51. Progressive disease of the circumflex artery and stable disease of the left anterior descending artery over a 27-month-period in a 41-year-old woman with angina, myocardial infarction between studies, and a risk factor score of 4. C: control study. 27 M: follow-up study after a period of 21 months. A tubular, regular grade 2 stenosis of the proximal circumflex artery (black arrow, left) progressed to complete occlusion. Short, concentric stenoses of the mid-left anterior descending artery remained unchanged (arrowheads). (From Rosch, J. et al., *Radiology*, 119, 513, 1976. With permission.)

with nonsignificant coronary atherosclerosis will not show progression of lesions over a 3- to 4-year period.[198]

- Twenty-five patients were restudied after an average of 12 months of optimal medical treatment. About 20% of significantly blocked arteries did worsen; all obstructions of greater than 90% at the first study were recorded as "total occlusion". In six of seven patients with these occlusions, an acute myocardial infarction occurred. As concerns the new complete occlusions, they were recorded in the vessels considered to have produced myocardial ischemia occurring as a transient ST-T change on electrocardiogram.[210]
- A prospective study was carried out on the coronary circulation in patients with acute coronary insufficiency (reversible ST-T changes, 80 to 95% anatomic stenosis). Angiograms repeated 4 months later revealed new occlusive atherosclerotic plaques in 9 of 30 severely stenotic vessels. Eight of the new occlusions occurred in severely narrowed arteries previously correlated with regional ST-T changes. Six patients had myocardial infarctions, five of whom corresponded with the site of a new occlusion.[211] Repeated coronary angiography may thus be used to provide indirect evidence that the acute coronary insufficiency syndrome could be related to intermittent, transient coronary artery occlusion and a threat of new permanent occlusion of the same artery. The intermittent coronary obstruction might result from reversible platelet aggregation or coronary spasm.
- The clinical course was followed for 10 years in 521 patients whose coronary arteriograms did not show any severe obstruction. Coronary events occurred in 2.1% of those who had normal arteriograms, in 13.8% of the group with mild stenotic atherosclerotic plaques, and in 33% of those with a moderate degree of coronary arterial narrowing. Gross survival of patients who were thought to have either normal arteriograms or less than 30% narrowing of a major coronary artery was 93.6% after 10 years, compared with 92.3% for the general population of the U.S. for the same interval. Survival was significantly lower (74.6%) for the subset of patients who had moderate obstructions, occurring as a 30 to 50% decrease in estimated diameter.[212]

- The evolution of coronary atherosclerotic plaques was analyzed in 256 patients who had significant lesions and in whom arteriography had been performed twice. The progression percentages increased proportionally with the degree of initial narrowing and the interval between the catheterizations, ranging from 1.2% to more than 20%. The incidence rate of presumed cardiac death after 5 years of follow-up was 2% in patients with stenosis of less than 30%, a rate lower than that observed for an average population matched for age and sex. The incidence rate of presumed cardiac death in patients with an intraluminal narrowing of more than 30%, but less than 50% was 5.3% after 5 years, slightly more than that observed in the average population.[213] The duration, character, and evolution of clinical symptoms do not seem to be helpful in separating cases showing angiographic signs of progression from those with an apparent stabilized disease. Furthermore, patients with a similar morphologic pattern of coronary arteriography may be divided into a subgroup which becomes severely ischemic and a subgroup with stenotic lesions well compensated by collateral vessels. The highest progression percentages were shown by the proximal segment of the major coronary arteries and also by the proximal segment of the first septal, first diagonal, and left marginal vessels. In 56.3% of the patients, progression was found in at least one vascular segment, ranging from 40% in patients who were recatheterized within 1 year to 92% in patients who were recatheterized after 5 or more years.[213]
- In a study of 262 subjects with stenotic coronary atherosclerotic plaques, 14%/year of the group was exposed to worsening of the lesions. The progression of lesions was more marked in subjects under 50 years of age and this progression did not seem to be significantly influenced by the major risk factors.[214]
- Repeated coronary angiograms of 58 patients were quantitatively evaluated. All patients had significant lesions at the time of the first angiogram and the second coronary angiogram was performed following aggravation of myocardial clinical manifestations (anginal symptoms in the majority of cases). In 29 patients coronary arteriograms were repeated after an average interval of 36 months and 42% of all measured segments remained unchanged. In 29 other patients, the average interval was 60 months and 42% of segments demonstrated unchanged diameters. Persisting unchanged coronary artery segments were distributed almost equally among the three major coronary arteries. Overall progressive lesions were found in 17 to 22% of all measured segments. Most progression was from an initially normal segment to a mild to moderate stenosis of less than 50%. In some vessels, such as the left main coronary artery, progression was not related to the interval of time between studies, whereas in other major coronary arteries the length of time seemed to be an important determinant of progression. A particular finding was the high rate of progression recorded in vessel segments with normal diameters measured at the first coronary angiographic examination. Almost 60% of progressive segments started from normal at the onset to a mild or moderate obstruction at the second angiographic examination.[215]
- A computerized edge tracking and lesion measurement in coronary angiograms was used in a pilot study comparing smokers to nonsmokers. The results demonstrated a more rapid progression of lesions in smokers — continued smoking accelerated growth of coronary atherosclerosis by 4%/year. When smoking was interrupted, the severity of lesion progression was lessened.[216]
- There were 300 patients who were studied by at least two catheterizations at intervals from 9 months to 13 years. The normal cases progressed less frequently than did patients with either mild narrowings or with severe stenotic lesions. The incidence of progression was similar in cases with mild or severe stenoses whereas individuals with angiographic normal coronary arteries rarely progressed to advanced plaques, and then only rarely within <4 years. The results suggest that even apparently mild stenotic plaques represent serious lesions which progress frequently in symptomatic patients to more advanced forms.[217]
- In an angiographic study carried out on 313 consecutive patients with coronary heart

disease treated medically, 33% showed a new occlusion at the second catheterization. After intervals of less than 2 years male smokers with at least 80% diameter stenosis were at higher risk of occlusion than the other patients. The presence of stenosis of 80% or greater jeopardizing an akinetic left ventricular segment appeared as a powerful predictor of subsequent occlusion. The appearance of a new occlusion can be predicted at the time of the first arteriogram based on the severity of stenosis (80% or greater luminal narrowing), extent of stenosis, smoking habit, male sex, and contractile capacity of the left ventricular area supplied by the stenotic vessel.[207]

- A clinical and arteriographic study was carried out on 52 patients with coronary heart disease who underwent repeated coronary arteriographies separated by 2 to 108 months of medical treatment. The lesions appeared to progress episodically in relation to the abrupt development of new symptoms of acute coronary events such as unstable angina or myocardial infarction. From the preexisting stenoses, 33% showed evidence of progression. Intimal hemorrhage appeared as the likely explanation for lesion encroachment and also for the episodic nature of the progression of coronary atherosclerosis. This progression is not linear and local anatomical factors appear to play a dominant role in their natural history. Favored sites are the right coronary artery around the origin of the right marginal and posterior descending branches, the left circumflex artery at the branch site of the left marginal vessel, and the left anterior descending artery above and below the origin of the main septal branch. All these branches fix the major coronary arteries to the myocardium. The progression of lesions at these sites appears to be unpredictable — frequently new lesions develop in previously normal sites. This progression showed little or no correlation with cardiovascular risk factors.[218]
- Progression was recorded in 16 or 31 patients with minimal atherosclerosis and in 3 of 20 cases with normal coronary arteries. By multivariate logistic regression, progression was predicted by number of disease segments, age, smoking habit, and initial cholesterol level. This study also revealed that patients with normal or minimally narrowed coronary arteries at angiography form a heterogeneous population including both normal and borderline subjects and patients with an early atherosclerotic involvement. Patients with advanced lesions and numerous risk factors were at high risk of progression; conversely, patients with normal coronary arteries and no risk factors were at low or very low risk.[208]
- Many studies revealed that in patients with bypass grafts, progression of the lesions can be detected by serial arteriograms in both grafted artery and in the nongrafted ones. Particularly in the grafted major coronary artery progression of the lesions seems to be significant, the use of repeated coronary angiography revealing than in many patients coronary bypass grafting accelerates the progression of preexisting atherosclerotic plaques toward occlusive lesions. This progression seems to be dependent on local hemodynamic changes, particularly stasis in the area of stenosis, followed by thrombosis. As concerns obstructions immediately distal to the anastomosis, this is mainly related to a technical consequence of the operation, rather than to the natural history of coronary atherosclerosis.
- An investigation performed on 34 patients at 13 and 61 months after surgery revealed progressive graft narrowing induced by atherosclerotic lesions in 44% of patients. Another investigation refers to sequential control angiographic examinations performed 2 weeks, 1 year, 5 to 7 years, and 10 to 12 years after saphenous bypass surgery. Changes attributed to atherosclerotic involvement were revealed after 1 year and the incidence of these changes increased from 16 to 36.4% during the interval between 1 year and 5 to 7 years. Graft closure augmented 2.5-fold from the interval between 1 and 12 years. Ten years after saphenous vein aortocoronary bypass surgery, of 132 grafts shown to be patent 1 year after surgery, only 50 were unaffected. Progression of atherosclerotic plaques in the coronary arteries without grafts was also frequent, both in vessels that were normal and in those with minor stenosis. New lesions did not develop in 15 patients, but did in 67 in the grafts, the

native vessels or both. In this group of patients, followed up for 10 years after surgery, several coronary angiographic examinations demonstrated that atherosclerotic plaque progression in both grafts and in the vessels without grafts is very common. Plasma cholesterol and triglyceride levels were significantly higher in patients with new plaque development and progression. Levels of high density lipoprotein cholesterol and plasma low density lipoprotein (LDL) apoprotein B also distinguished the group without and with significant progression of atherosclerotic lesions.[219-221]

Repeated coronary angiography was also used to demonstrate *regression* of atherosclerotic lesions.[210,213] A study dealing specifically with regression aspects, limited to patients with severe hypercholesterolemia and severe coronary atherosclerosis before and after partial ileal bypass revealed definite evidence of regression in 13% of patients.[222] Partial regression of coronary atherosclerosis was also reported in two homozygotes and in one heterozygotic patient with familial hypercholesterolemia.[223] Concomitantly, with these observations on the regression of stenotic lesions it was possible to record that progression of certain atherosclerotic plaques could be partially inhibited by effective treatment of hypercholesterolemia. Some changes in the lesions strongly suggest that the natural history of coronary atherosclerosis can be altered and that certain plaques are potentially reversible. Optimistic views on retarding progression and producing regression came from studies on the effects of cholestyramine and a low-fat, low-cholesterol diet. A bile acid sequestering agent such as cholestipol hydrochloride might also induce regression in patients with hypercholesterolemia. On the other hand, we were unable to find in the available literature data on the regression of atherosclerotic plaques on successive arteriograms following the reduction of blood pressure levels. A possible source of error in all studies on the regression of coronary atherosclerosis is the tendency of vascular segments to become enlarged with age. An arterial dilation can occur without a concomitant change in the size of the lesion and in its stenotic character, and this may be interpreted in a second coronary angiogram as a regression of atherosclerotic involvement. This is the reason many investigators consider that atherosclerosis regression recorded solely on luminal changes may be misleading. We must also caution against the tendency to speak of the regression of advanced atherosclerotic plaques when occlusive mural thrombi decrease in size or disappear on sequential arteriograms. Lysis, contraction, or recanalization of a thrombus cannot be taken to represent regression of an atherosclerotic plaque.[214] Also of note is that angiographically determined regression may be stimulated by relaxation of spasm, medial atrophy, or arterial ectasia.

Regression, as seen on two successive coronary arteriograms may reflect a reduction in both intra- and extracellular lipid of the plaque, in cell mass, and in necrotic areas. The return to a normal state of the arterial wall is difficult to accept. An additional test of regression may be the disappearance of collateral vessels.[225] Such a disappearance was revealed after 2 years of lipid-lowering therapy by diet and cholestipol.[226] In essence, repeated coronary angiography is the best available method to record regression in human coronary arteries involved with severe atherosclerotic lesions. The use of computer-estimated atherosclerosis revealed more patients with lesion regression than did visual estimation. A computer index of atherosclerosis severity can be obtained by measuring irregularity of vessel shadow edges and densities. Computer measurements made it possible to estimate some local changes in each individual patient's vessel and to correlate the direction and rate of change with risk factors and various treatments.[227] Regression can be assessed prevalently in an anatomic or a functional sense. Anatomic regression is particularly measured by changes in percentage of narrowing, absolute diameter, and length and shape of the lesion; functional regression is measured particularly by the changes in coronary blood flow, pressure gradient, distal perfusion pressure, and coronary flow reserve. It is obvious that visual estimation cannot cover all these complex changes in order to demonstrate the regression of atherosclerotic plaques.[151]

E. Visualization of Coronary Collateral Vessels

1. General Observations

In the second half of the 19th century, Conheim (1881) and other investigators assumed that coronary arteries are end arteries. This view was repeatedly challenged by the results of many important studies which demonstrated the presence of collateral vessels in the human heart.[2,6,23,53,187,228-240] Moreover, communication between the coronary arteries and the mediastinal vessels were visualized by many investigators, numerous anastomoses being revealed between bronchial arteries, the pericardophrenic branches of the internal mammary artery, the anterior mediastinal, pericardial, superior and inferior phrenic, intercostal, esophageal and other branches of the aorta, on the one hand, and the coronary circulation, on the other hand. This rich, potential extracardiac coronary collateral circulation is of particular significance; anastomoses between bronchial and coronary arteries with a diameter of more than 2 mm are visualized in in vivo angiograms.[237] In addition to the anastomoses between coronary arteries and mediastinal vessels, of particular importance appeared to be those between the left and right coronary arteries, demonstrated at the beginning of this century by means of stereoarteriography.

All of the above-mentioned anastomoses are universal and abundant and can be clearly visualized in living subjects with the aid of coronary angiography.[239-240]

Unfortunately, in spite of so numerous and clear proofs that coronary arteries are not end arteries, this last view still persists and even in very recent cardiology textbooks one can read that coronary arteries are end arteries. Obstruction anywhere in these vessels will certainly cause myocardial infarction somewhere in the heart. On the other hand, these textbooks overlook the fact that in any area of the normal heart, countless communications are present among coronary segments of various size. Many of these anastomotic vessels have a diameter of 10 to 50 μm and are not visible on coronary angiography; other intercoronary anastomoses with a diameter over 100 μm become visible on an adequately performed coronary arteriography.

The available literature demonstrates that in spite of continuous technical improvement of the coronary arteriography technique, it is still limited as regards information of the real extension, size, and functional significance of collateral vessels.[232] Therefore, only data obtained on post-mortem material by means of plastic casts are of particular value (Figure 52).

The inability of coronary angiography to detect the smaller or deeper collateral vessels does not mean that such vessels do not exist or are not functionally present. This error is frequently made in the studies published in the available literature: the atherosclerotic lesions do not exist if they are not visualized by gross inspection, the collateral vessels do not exist if they do not appear on coronary angiograms. Plastic casts clearly demonstrate that coronary collateral vessels' satellite to an obstructive atherosclerotic plaque may include a large number of relatively small channels not visible by angiogram and only a small number of relatively large vessels which may become visible.

After decades of controversy it now appears that small, medium, and large collateral vessels are able to carry substantial volumes of blood and are often able to preserve normal resting coronary blood flow to the territory of a totally or nearly totally occluded major coronary artery. Even in the setting of increased myocardial oxygen demand, as with exercise, some collaterals are sufficiently large to continue to supply the necessary increase in flow. It is thus possible for certain collateral vessels to supply a myocardial area like a major coronary artery. We have, like many other pathologists, frequently found on post-mortem examination occluded or obstructed coronary arteries coexisting with an apparently normal myocardium due to the presence of an adequate collateral circulation. For similar reasons most people with one or more severe stenoses on coronary angiograms are asymptomatic and for similar reasons infarct size does not relate to the number and severity of coronary

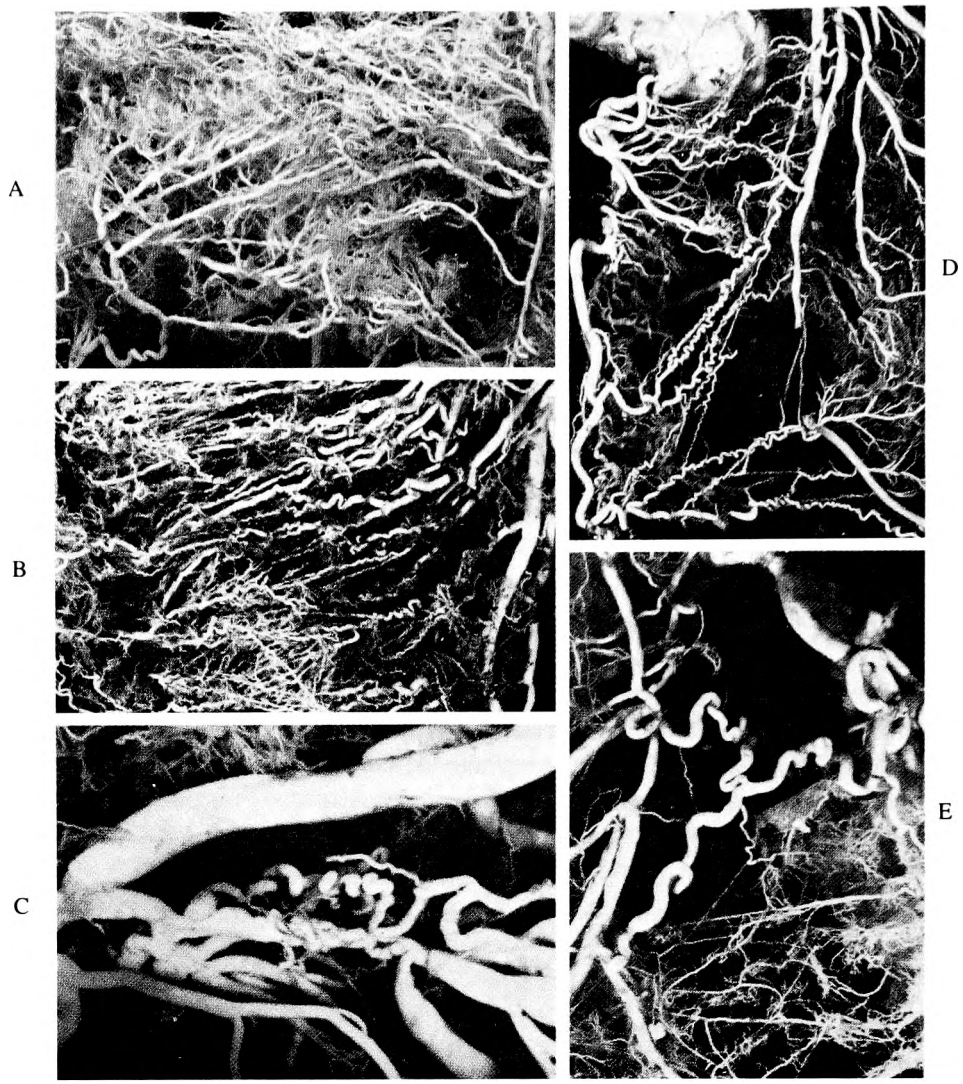


FIGURE 52. Different patterns of highly enlarged anastomotic circulation in human cases of coronary occlusion. (A) Posterior; (B) septal, intercoronary anastomoses. Compare the high frequency of small vessels and the low frequency of enlarged ones (E). (C) "Wall" collateral between proximal and distal portion of an occluded vessel. (D) "Satellite anastomotic circulation" in a case of double occlusion of the left anterior descending artery. Note the sharp relation between the collateral sources and the occlusions. (From Baroldi, G., *Acta Cardiol.*, Suppl. 13, 7, 1969. With permission.)

occlusions or narrowings. It has been shown, for instance, that 66% of patients dying in the hospital from various noncardiac disease and 39% of normal, healthy subjects dying by accident had one or more severe coronary stenoses, narrowing more than 70% of the coronary artery lumen.[241] It is tempting to presume that in all these cases unrecognized collateral vessels have limited or even eliminated the consequences of ischemia produced by stenotic plaques. A stenotic lesion which has reached the point of critical narrowing for the production of myocardial ischemia may not be of potential clinical significance if a compensatory development of collateral vessels exists (Figure 53).[239]

Many clinicians and particularly many surgeons are firmly convinced that the presence of collateral vessels is an important event in the natural history of coronary atherosclerosis. This view stimulated certain experimentalists and pathophysiologists to investigate whether myocardial ischemia, infarction, and other complications could be prevented if we could enhance the development of numerous and large collateral vessels. Treatments capable of

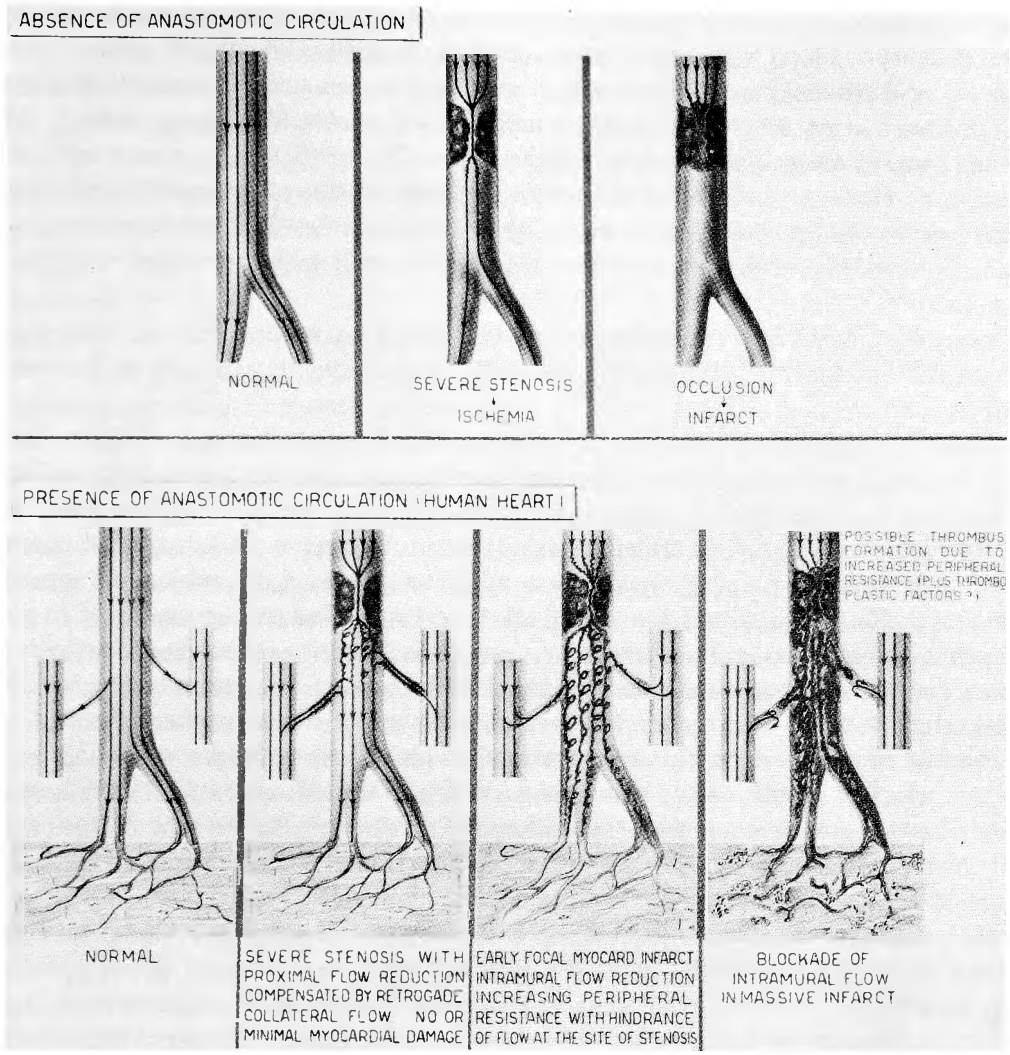

ABSENCE OF ANASTOMOTIC CIRCULATION
NORMAL
SEVERE STENOSIS
ISCHEMIA
OCCLUSION
INFARCT
PRESENCE OF ANASTOMOTIC CIRCULATION (HUMAN HEART)
POSSIBLE THROMBUS FORMATION DUE TO INCREASED PERIPHERAL RESISTANCE (PLUS THROMBOPLASTIC FACTORS ?)
NORMAL
SEVERE STENOSIS WITH PROXIMAL FLOW REDUCTION COMPENSATED BY RETROGADE COLLATERAL FLOW NO OR MINIMAL MYOCARDIAL DAMAGE
EARLY FOCAL MYOCARD INFARCT INTRAMURAL FLOW REDUCTION INCREASING PERIPHERAL RESISTANCE WITH HINDRANCE OF FLOW AT THE SITE OF STENOSIS
BLOCKADE OF INTRAMURAL FLOW IN MASSIVE INFARCT

FIGURE 53. Hemodynamic changes in obstructive arterial disease in the absence or presence of anastomotic circulation. (From Baroldi, G., in *Methods and Achievements in Experimental Pathology*, Vol. 5, S. Karger, Basel, 1971. With permission.)

stimulating the onset of functionally adequate collateral vessels might help to reduce the morbidity and mortality from coronary heart disease and hence the cost of coronary bypass operations.[234] This surgical bypass is compared to a collateral flow at very high pressure.[6,241] In a group of asymptomatic patients with heterozygous familial hypercholesterolemia, the hypocholesterolemic therapy stimulated the development of angiographically visible collateral vessels. This led to the conclusion that vigorous, antilipemic therapy in patients with advanced coronary atherosclerosis may facilitate collateral vessel development.[242]

Apparently, for genetic reasons, certain subjects have a more adequate anastomotic potential than other subjects. This genetic predisposition to develop coronary collaterals was more clearly demonstrated in laboratory animals and appeared highly variable being viewed as a continuum: sheep represent one extreme of this continuum being unable to develop collaterals; at the opposite extreme is the dog which may develop large epicardial collateral vessels after gradual occlusion of a major coronary artery. Since dogs may form extensive epicardial collaterals, gradual closure of a major coronary artery in the canine model rarely results in myocardial infarction. In certain experimental conditions collateral flow in dogs approximated 40% of normal left anterior descending artery flow at the same perfusion

pressure.[243] These results clearly show that collateral vessels may provide an alternate route for the delivery of blood to the myocardium distal to obstruction of a major coronary artery. Pigs have only a subendocardial network of collateral vessels and the gradual closure of a major coronary artery invariably results in myocardial necrosis. The rhesus monkey shows a limited capacity to develop coronary collaterals, usually insufficient to prevent myocardial infarction. In essence, studies dealing specifically with the size of myocardial infarction in various species clearly demonstrated that there is a wide species-dependent spectrum in the extent of infarction and that these differences correlate well with the volume of collateral blood flow.[244]

In man, three basic factors might influence the size of myocardial infarction: the size of the occluded vascular bed, the number and caliber of collaterals available at the time of occlusion, and the rate of development of new collateral vessels.[245] This rate of collateral development stimulated by gradual narrowing produced by an obstructive atherosclerotic plaque is critical in limiting infarct size, since it will determine the status of the collateral circulation at the time of total occlusion. The infarct size is usually smaller in gradual vs. acute occlusion and continued collateral development may serve to increase the coronary reserve to the remaining viable myocardium. Additional data on this subject are presented in Chapter 5. They demonstrate that collateral vessels may play an important role in maintaining myocardial function at rest and in preventing or minimizing infarction and arrhythmias in some patients with severe stenotic atherosclerotic plaques. If collateral vessels had no influence on the course of events during the natural history of coronary atherosclerosis, then every area of the myocardium supplied by an artery with an obstructive plaque would become infarcted, which is not the case. On the contrary, due to the existence of collateral vessels, pathologists often visualize at necropsy myocardial infarcts smaller than the perfusion area of the obstructed major coronary artery and even cases of total occlusion with no myocardial damage.[246,247]

There is at present no animal model which predictably develops coronary collateral vessels sufficient to prevent myocardial infarction after coronary occlusion and whose collaterals are in an anatomic location similar to that in man.[248] Only some dogs have sufficient collaterals to prevent infarction following gradual occlusion of the left circumflex artery or the right coronary artery, but these collateral vessels exhibit a prevalent epicardial topography.[249] Nevertheless, the canine experimental model is of particular importance, since collateral vessels develop 6 months after coronary occlusions are able in some animals to replace the occluded artery almost completely from a functional point of view.[250] The canine heart is also adequate for demonstrating that collateral resistance decreases remarkably during slow progressive coronary artery stenosis up to complete occlusion.[251]

Large collateral vessels were visualized to appear transiently during prolonged coronary spasm. In patients with angina pectoris who showed isolated spasm in the proximal segment of the left anterior descending artery during ergonovine provocation testing, the development of collateral vessels was associated with a lesser degree of myocardial ischemia and necrosis, compared with the cases not supplied by collaterals.[252]

2. Collateral Vessel Distribution

There is convincing evidence that *in human hearts numerous vascular channels are interposed as connecting links between major coronary arteries and main branch vessels capable of bypassing the area of coronary occlusion.*

The extent to which collateral vessels may assume a certain part of the role of these major coronary arteries and main branch vessels can be related to: (1) the genetically dependent development of collateral vessels, (2) the pattern of myocardial distribution of the performed collaterals, (3) the genetically dependent anatomical branching pattern of the coronary arteries, (4) the existence of one or of several stenotic lesions in the coronary arterial tree,

(5) the location of the obstructions, (6) the rapidity with which these obstructions occur, and (7) the metabolic requirements of the myocardium.

When an acute occlusion appears, the fate of the myocardial area supplied by the occluded vessel depends upon the cross-sectional area of the preexisting bridging collaterals.

When a chronic occlusion develops over months or years, progressive adaptation of the collateral vessels can be detected and these anastomotic channels may reach a final stable level of variable significance. Angiographically recorded collaterals are considered able to significantly reduce the magnitude of exercise-induced ischemic dysfunction in regions of the left ventricle supplied by stenotic coronary arteries. Collateral vessels can even protect against exercise-induced myocardial perfusion defects, as detected by thallium scintigraphy.[236] Such improvements in myocardial perfusion are considered to have a beneficial effect on left ventricular function.

Based on the results of experimental models and of post-mortem investigations on human hearts, it might be presumed that the relationship between an area of the myocardium supplied by an occluded artery and the infarction area developing in the region concerned is largely influenced by the distribution and availability of collateral vessels. A potential source of anastomotic compensation exists for each stenotic lesion, since such lesions usually have their satellite anastomotic channels. This anastomotic reserve strongly influences the natural history of coronary atherosclerosis, accelerating or retarding myocardial ischemia, and its clinical consequences. The available literature suggests that enlargement of the normally well-developed intracardiac arterial anastomotic channels appears minimal when luminal narrowing is less than 50% and is two- or threefold when stenoses rise to 75%, whereas a maximum development is present when the vessel is totally occluded.

A potential source of anastomotic compensation is present in all the regions of the heart. Beyond a critical point of lumen reduction, marked enlargement of the collaterals takes place which becomes maximum in the presence of multiple occlusions, each exhibiting its satellite collateral vessels. The following areas of the heart are particularly rich in collateral vessels: apex, septal wall, anterior surface of the right ventricle, left and right atria, and the crux cordis. This distribution is considered to be influenced by the presence of right or left predominant circulation, collateral vessels being more frequently visualized at the apex in the presence of predominant left circulation and at the crux cordis in the presence of predominant right circulation. However, the relation between predominant circulation and collateral development remains obscure, the so-called predominance of the left or right coronary circulation being considered a misguided concept by many authors.[2,23,33,71,253]

The natural history of coronary atherosclerosis can be influenced by the preexistence and ulterior development of both homolateral and heterolateral anastomoses.

The communications which exist between two major coronary arteries are considered heterocoronary or intercoronary anastomoses; when communications appear between the segments of the same vessel or/and branches of the same major coronary artery they are called homocoronary or intracoronary anastomoses. Both types of vessels may join all regions of the coronary arterial tree. In order to visualize the topography of collateral vessels, it is first necessary to establish the anatomical branching pattern in each case studied. Along the course of the left anterior descending artery, the most common order of origin of branches is the first diagonal, the first septal, the first right ventricular, the second and third septals, and the second diagonal. The diagonal and septal arteries must be identified in the order of their origin and the vessel greatest in size is called "main". Main branches are usually the first septal artery and the first or second diagonal branches.

From the left circumflex artery a main branch, the left (obtuse) marginal vessel, arises, but it is also possible to visualize angiographically a second or third marginal branch.

Some pitfalls in interpretation may occur when the above-mentioned vessels have peculiar origins (the septal artery may originate from the diagonal vessel or from the right coronary

artery, the posterior descending vessel from the left marginal branch, etc.). It is also very difficult to record the presence of collateral vessels and their origin when these vessels arise from many different sources. Thus, for instance, in patients with chronically occluded left circumflex artery collaterals arise from branches of the second and third order of the left anterior descending artery, the septal vessels, the right coronary artery, the sinus node vessel, and from small branches which traverse the atria.[250] It is also difficult to reveal angiographically collateral vessels which exist along the intramural course of many branches. Along this intramural course, a real anastomotic network of collateral vessels can be detected in post-mortem casts which has no correspondence on coronary angiograms. This might be an invitation for many angiographers to use the term "angiographic absence" of collateral vessels and not to use the term "absent" in an absolute sense since this may represent the source of mistaken conclusions.

The coronary angiographer may visualize:

1. Left homolateral anastomoses arising from the septal and diagonal vessels and their smaller branches between the proximal and distal segments of the left anterior descending artery
2. The anastomoses between the left marginal and diagonal vessels
3. The anastomoses between left circumflex artery and left atrial branches
4. Homolateral anastomoses occurring as right atrial vessels and right marginal vessels, all able to connect the proximal and distal segments of the right coronary artery
5. A right marginal branch connecting the right coronary artery before the crux cordis with the posterior descending vessel
6. Heterolateral anastomoses from the right coronary artery to the left anterior descending artery or vice versa, commonly seen as the terminal network of the septal vessels connecting the left anterior descending and right posterior descending arteries
7. Connections by means of the conus branch, the right and left marginal vessels, Kugel's artery, the sinus node artery, and the atrioventricular node artery

These last connections are of particular importance, being demonstrated by angiographers in cases with obstruction of the left main coronary artery, without severe myocardial clinical manifestations. Selective angiographic examination of the coronary arterial tree revealed a well-developed right coronary artery giving rise to extensive collateral vessels permitting opacification of both left anterior descending and left circumflex arteries.[254]

An illustraive example was selected to demonstrate the complexity of anastomoses which develop in the presence of stenotic atherosclerotic plaques.[255]

When more than 90% narrowing existed in the right coronary artery, the following development of collateral vessels was revealed.

1. The left anterior descending artery with the right posterior descending artery via the septal branches
2. The terminal segment of the left circumflex artery with the terminal segment of the right coronary artery
3. The left marginal branch of the left circumflex artery with the posterior left ventricular branch of the right coronary artery
4. The proximal segment of the right marginal branch or conus branch of the right coronary artery with the terminal segment of the left marginal branch
5. The proximal and distal segments of the right coronary artery by means of Kugel's artery
6. The distal segment of the left anterior descending artery with the distal segment of the right posterior descending artery around the cardiac apex

7. The distal left circumflex artery with the right coronary artery by means of the atrioventricular node branch
8. The right marginal branch of the right coronary artery with the posterior descending branch of the same right coronary artery via the diaphragmatic surface of the right ventricle
9. The sinus node branch of the right coronary artery with the atrial branches of the left circumflex artery via the lateral wall of the left atrium
10. The branches to the right ventricle of the left anterior descending artery with those of the right marginal branch of the right coronary artery[255]

In patients with severe narrowing or complete obstruction of the left anterior descending artery, seven pathways of collateral circulation were delineated:

1. The right marginal branch of the right coronary artery was connected with the left anterior descending artery
2. The proximal septal branch appeared connected with the distal septal branches of the same left anterior descending artery
3. The left marginal branch of the left circumflex artery occurred connected with the terminal branches of the left anterior descending artery
4. The conus branch of the right coronary artery was a source of anastomotic vessels for the left anterior descending artery
5. The diagonal branches appeared also interconnected with the distal segment of the left anterior descending artery
6. Many interventricular septal branches connected the posterior descending artery with the left anterior descending artery[255]

When severe stenoses or complete obstructions were present in the left circumflex artery, the following pathways of collateral circulation were delineated:

1. The left atrial circumflex branch with the terminal segment of the left circumflex artery
2. The proximal segment of the left marginal branch of the left circumflex artery with the terminal segment of the same vessel
3. The diagonal branch of the left anterior descending artery with a left marginal branch of the left circumflex artery
4. The terminal segment of the right coronary artery with the terminal segment of the left circumflex artery
5. The posterior left ventricular branch of the right coronary artery with the left marginal branch of the left circumflex artery[255]

Diagrams of Figures 54, 55, and 56 illustrate these specific anastomotic pathways.

The existence of radiologically invisible anastomotic channels between the major coronary arteries and the major branch vessels is no longer doubtful. Similarly, the ability of some collateral vessels to become large enough to be visualized angiographically is no longer questioned. Some of the collateral vessels have diameters of 1 to 2 mm, but they are demonstrable in coronary angiography when their lumen is more than 0.1 mm.[256,257] There is a collateral vessel potential that exists in each heart and which must be carefully investigated.[258-269] Of particular importance are cases with total coronary occlusion and absence of myocardial damage and cases in which the myocardial infarct size is significantly smaller than the perfusion area of the obstructed major coronary artery.

When angiography is adequately performed, collateral vessels appear as anastomotic channels with a clear point of origin, a mid-zone, and a reentry point.[262] The operator must

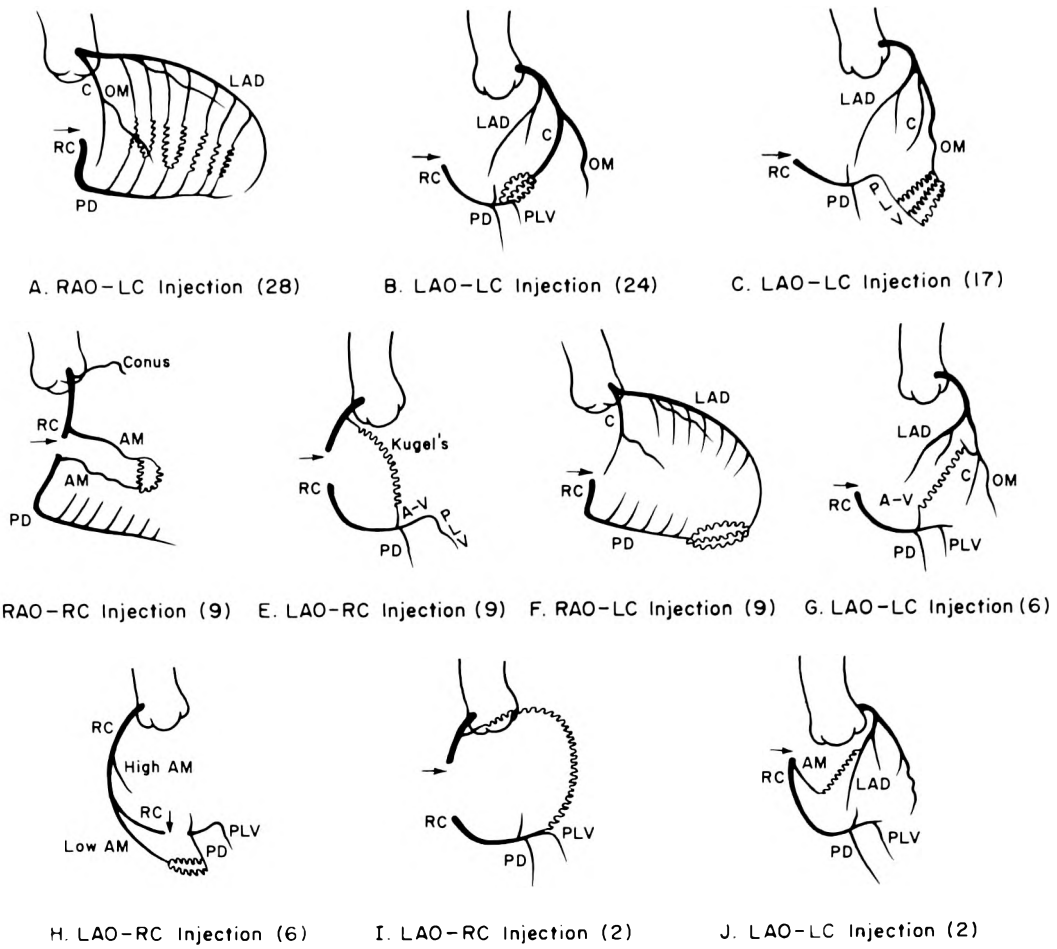


FIGURE 54. Collateral pathways in right coronary artery (RC) obstruction, RAO: right anterior oblique projection. LAO: left anterior oblique projection. LC: left main coronary artery. AM: acute (right) marginal branch of the right coronary artery. PD: posterior descending branch of the right coronary artery. PLV: posterior left ventricular branch of the right coronary artery. A-V: artery to the atrioventricular node. LAD: left anterior descending artery. C: circumflex artery. OM: obtuse (left) marginal branch of the circumflex artery. Numbers in parentheses signify frequency with which the given pathway occurred in this series. (From Levin, D. C., *Circulation*, 50, 831, 1974. With permission.)

ensure adequate filling of the whole coronary arterial bed with contrast medium to be certain that collateral vessels have not been overlooked. This circulation is better and more completely visible after administration of nitroglycerine which may suppress a spasm, produce distal vasodilation, reduce intramyocardial pressure, and may also influence the transstenotic pressure gradient.

Difficulties in interpretation are produced by the occurrence of retrograde and antegrade currents, as well as by images of back flow at the junction points. Difficulties in interpretation are also due to the fact that if the obstruction is removed some collateral channels again become nonfunctional.[263] Collateral channels that had not been visible on the preoperative angiogram were demonstrated postoperatively in those patients in whom graft occlusion was associated with a new complete obstruction of the recipient artery. If antegrade flow had been restored by a functioning graft, collateral channels that filled before surgery were no longer visible.[264] Many other reports have emphasized the reappearance of collateral channels that had been present preoperatively but not visualized after initially successful vascularization, if occlusion of the graft developed. These observations support the view that in humans, the coronary collateral circulation can appear and regress rapidly according to

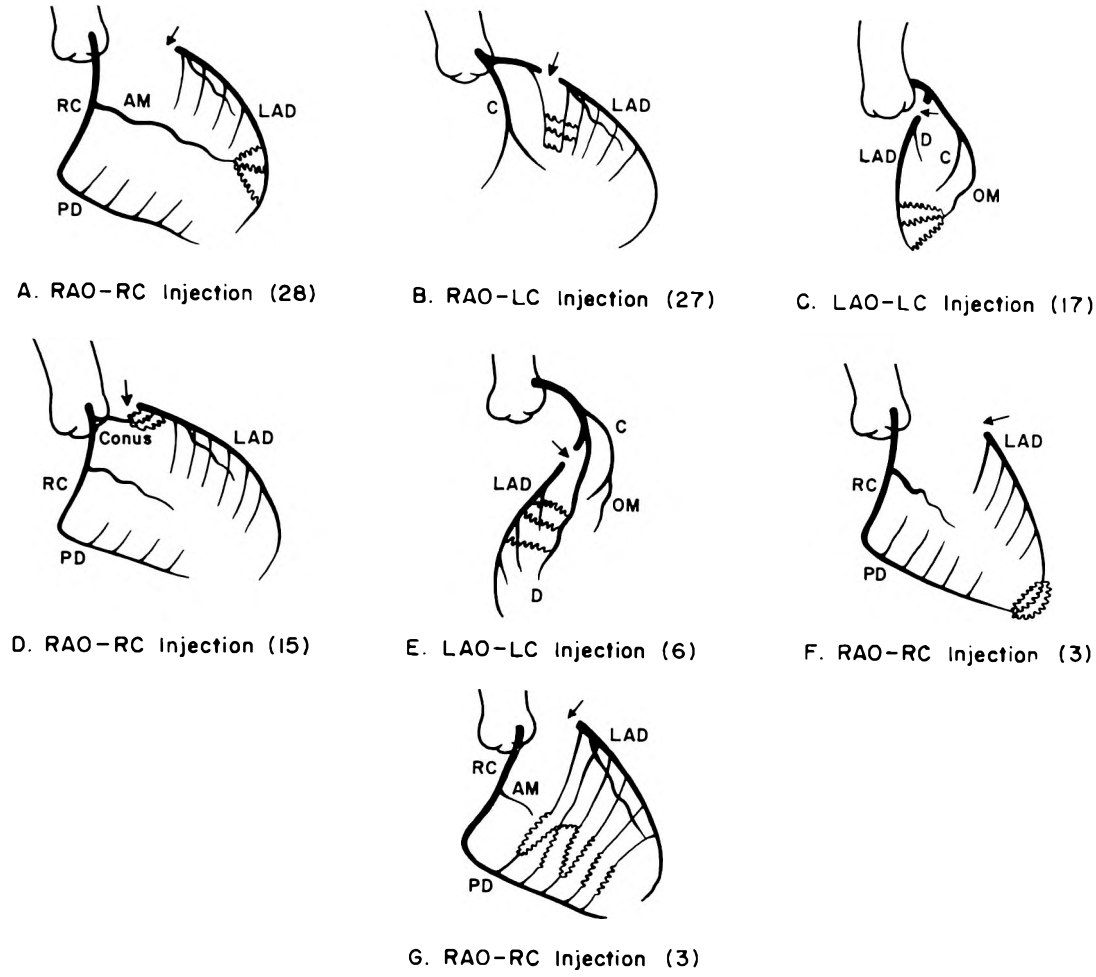


FIGURE 55. Collateral pathways in left anterior descending artery obstruction. D: diagonal branch of the left anterior descending artery; other abbreviations as in Figure 54. (From Levin, D. C., *Circulation*, 50, 831, 1974. With permission.)

myocardial nutritional needs and pressure gradients. The occurrence of such vessels is regarded by many investigators as an active process and not as a passive stretching of preexisting channels, being arbitrarily divided into well-defined stages of development.[249] The majority of developmental activity seems to occur in the mid-zone of preexisting collateral vessels; this zone is submitted to acute dilation and stretching, rupture of the internal elastic membrane, thinning of the muscular media, and perivascular inflammation. In a subsequent stage, medial smooth muscle cell proliferation becomes prevalent and the luminal diameter increases significantly.

The functional capacity of the coronary collateral circulation does not seem to be augmented by left ventricular hypertrophy.[265]

Angiographic visualization of collaterals provides little insight into the dynamics of vessel formation and function. Improvement of our present possibilities is expected from studies which use transluminal coronary angioplasty as a model for controlled coronary artery reperfusion, associated with changes in collateral vessel filling in patients with severe stenotic plaques and extensive atherosclerotic involvement.[266]

Clinical, electrocardiographic, and hemodynamic evidence showed that transient myocardial ischemia occurs less often and flow and pressure indexes of collateral perfusion are better in patients with than in those without angiographic evidence of an adequate network of connecting channels.[267] Certain investigators believe that in patients with coronary heart

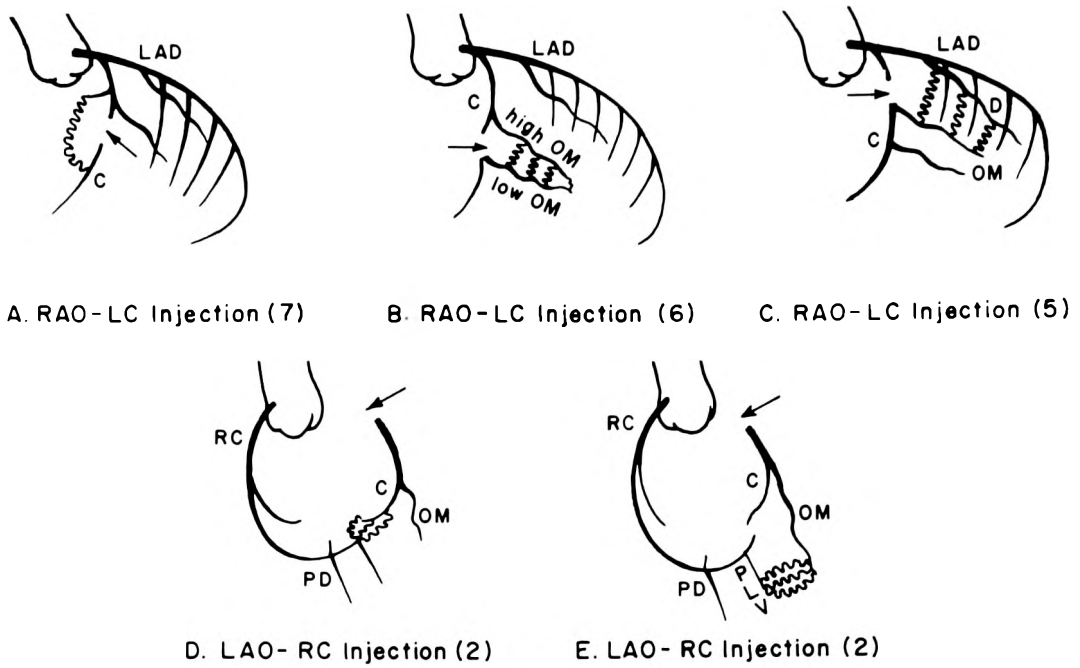


FIGURE 56. Collateral pathways in circumflex artery obstruction. Abbreviations as in Figure 54. (From Levin, D. C., *Circulation*, 50, 831, 1974. With permission.)

disease collateral vessels can prevent myocardial ischemia. According to other investigators the presence of well-developed collateral vessels can be interpreted as a reliable sign of myocardial perfusion deficit and of myocardial ischemia. There is much to suggest that in the human heart, coronary collateral vessels function in the same way as mild to moderate stenoses of the coronary artery: they cannot offer the flow available to meet increased myocardial demands.

A last and peculiar observation related to coronary vessel distribution refers to the differences in collateral vessel development occurring in patients with apparently similar degrees of vessel obstruction in the left and right circulation. In such patients collateral vessels were visualized angiographically in 53% of the right coronary artery critical stenoses and in only 29% of the left anterior descending artery critical stenoses.[258] On comparing similar severe stenoses in the right coronary artery and in the left anterior descending artery, the former stenoses were significantly more often bypassed by angiographically visible collaterals than the left anterior descending stenoses. It was also observed that the right coronary artery received intercoronary collaterals more frequently than the left anterior descending artery.[259] An apparently similar blood supply from collateral vessels prevents ischemic ST-changes during treadmill stress tests more frequently in the posterior than in the anterior left ventricular wall.[260,261] These results suggest not only that critical stenoses are less severe but also that the development of collateral vessels occurs at a lesser degree of stenosis in the right coronary artery than in the left anterior descending artery.

III. EXPERIMENTAL MODELS

A. Introductory Remarks

Hemodynamic stress, heredity, cholesterol, diet, environment, hypertension, cigarette smoking, psychologic stress, thrombogenic factors, growth factors, mitogens, viral and immune injuries, and carcinogenic agents are all related to the pathogenesis of atherosclerosis, considered a plurietiologic disease, and the arterial wall the target tissue for these multiple factors.

An experimental analysis of atherosclerosis as a multifactorial disease would require the use of a multitude of models in an attempt to define, delineate, and control the action of each particular atherogenic factor.

Of the mammals, man is unique in having a very high incidence of coronary atherosclerosis and a diffuse atherosclerotic involvement of extramural coronary arteries. Man is also unique in having stenotic atherosclerotic plaques, as well as ulcerated and thrombosed plaques, associated with myocardial clinical manifestations occurring as angina pectoris, myocardial infarction, and various types of severe arrhythmias followed by sudden cardiac death. In other mammals a similar spontaneous pathology of the coronary arterial tree and the myocardium and its conduction system cannot be encountered, the spontaneous developed atherosclerotic plaques very rarely encroaching upon the arterial lumen.

Owing to these peculiar aspects of human coronary atherosclerosis, a variety of experimental models have been developed for the study of the natural history of lesions, but none of them duplicates the pathologic feature, severity, and complexity of human coronary atherosclerotic involvement. This led to the conclusion that "the principal difficulty in the study of experimental atherosclerosis is the lack of an animal model that truly resembles man".[270] Moreover, as is true in all animal experiments, extrapolation of results across species lines is not justified as a logical process, since investigators cannot maintain that experimental findings in one species will be valid in a population of another species.[271] Likewise, certain scientists consider that "it is ironic that most experimental atherosclerosis and most arterial wall cellular and molecular biology are based on the observations on the aorta rather than the coronary arteries".[272] Indeed, no vessel or vascular region has a typical response with regard to the development of atherosclerotic lesions. Each arterial segment seems to be unique in its reaction to atherogenic agents and to various types of injuries, so that any one region cannot be used as a suitable model for another.

The following important characteristics would be required for an ideal animal model of human coronary atherosclerosis:[273]

1. The animal should reproduce in a laboratory setting and should present well-defined genetic characteristics
2. In the arteries, lesions should develop naturally while the animal consumes a reasonable diet
3. Lesions should develop slowly over the animal's lifetime with clinical sequelae in late middle or old age
4. Lesions should range from early to advanced complicated plaques, with superimposed thrombosis and severe stenosis
5. Complicated lesions should be more frequent in males than in females
6. The lesions should develop first in the aorta, then in the coronary arteries, and finally in the arteries of the brain

Since no single model fulfills all these requirements, it became necessary to use several models to investigate various aspects and stages of the natural history of human atherosclerosis with the experimentalist's attention usually focused on the early stages of atherosclerotic involvement. On the other hand, we are still far from an experimental model in which the end stages of the natural history of coronary atherosclerosis can be reproduced by means of stenotic and occlusive plaques complicated by thrombi, or in which the resulting myocardial infarct should exhibit a topography that would correspond to the territory known to be supplied by the occluded coronary artery. Here also, infarction should be obtained with a fairly high frequency and repeatability.

The limitations imposed by the absence of such models are very important, since we are not able to adequately investigate the hemodynamic changes produced by advanced, com-

plicated atherosclerotic plaques and their relation with angina pectoris, myocardial infarction, and sudden cardiac death. The possible roles of coronary spasm, platelet aggregates in intramyocardial vessels, or other types of microcirculation disturbances as related to coronary heart disease are also overlooked by the usual experimental models.

The difficulty in obtaining suitable models for the study of the natural history of coronary atherosclerosis and particularly of its end stages is augmented by the fact that some human risk factors, for instance those related to the highest functions of the nervous system, may not be reproducible in animals. A particular behavior pattern, the so-called type A personality[274] has no correspondent in laboratory animals and is involved in the pathogenesis of coronary heart disease. The impact of behavior and psychologic states on catecholamine release, blood pressure, platelet aggregation, serum fatty acid level, blood clotting mechanism, endocrine constellation, and sympathetic nervous activity can also be related to the pathogenesis of coronary heart disease. This was demonstrated in many publications showing that any behavioral predisposition to intense, frequent, and sustained central nervous system arousal may act as a risk factor for angina pectoris, myocardial infarction, or sudden cardiac death (cases with chronic anxiety, neurotic conflicts, depressions, sleep disturbances, etc.). In addition, the social position, social changes, life changes, life dissatisfactions, etc. may also act as risk factors for coronary heart disease.[275]

We are thus obliged to consider that even the most suitable models available for the study of the natural history of coronary atherosclerosis must be regarded only as simplified or even oversimplified forms of a very complex disease of multifactorial origin.

In the presence of an experimental model in which stenotic atherosclerotic plaques can be produced at will, we are also obliged to evaluate the availability of the collateral circulation. In man, as well as in experimental models, the existence of an adequate collateral circulation may completely eliminate the ischemic consequences of coronary luminal narrowing. The presence or absence of these collateral vessels and their functional adequacy cannot be determined in many experimental models. The aim of the experimentalist dealing with the natural history of coronary atherosclerosis is not to produce a stenotic lesion in a major coronary artery, but a stenotic lesion which is not bypassed by collateral vessels. When these collateral vessels exist, the magnitude and state of their development, associated with the severity of the stenotic or occlusive lesion, will determine the proper balance between myocardial oxygen supply and demand. Thus, caution is necessary not only in comparing different experimental models, but also in the extrapolation of information obtained to human coronary atherosclerosis and coronary heart disease.

Experimental information is often needed to supplement data which cannot be obtained from man, but some lesions that form in experimental models may be significantly different in their morphologic feature from those of human coronary atherosclerosis; likewise, the conditions required for the onset and progression of these lesions may also differ.

Experimental atherosclerosis is a term usually attributed to an oversimplified model to produce a multifactorial disease. This model is based on the aggravation of a major risk factor for coronary heart disease: hypercholesterolemia. There are no papers in the available literature that demonstrate that hypercholesterolemia is also able to produce atherosclerotic lesions in human coronary arteries or that these lesions do not appear in the absence of hypercholesterolemia.

No free living animals, including nonhuman primates, develop myocardial clinical manifestations induced by stenotic or occlusive atherosclerotic plaques complicated by thrombosis. On the other hand, coronary atherosclerosis is not a "privilege" of man; its spontaneous form is a general pathologic entity which can be studied in domestic animals. as well as in a variety of free and captive wild animals.

Many experimental aspects of early lesions might be considered inflammatory reactions, and cortisone, as well as other steroid and nonsteroid anti-inflammatory drugs retard the

onset of such lesions, acting as antiatherogenic agents. They can mitigate the arterial injuries produced by hypercholesterolemia without affecting plasma cholesterol levels.[276] Such particular antiatherogenic effects are not superposable on those detected in human disease and for this reason anti-inflammatory drugs are not used to prevent the onset of early atherosclerotic lesions in human coronary arteries.

In experimental models, cholesterol and its esters are able to induce a chronic inflammatory, organizing, or even sclerosing response when injected into vascularized connective tissue. A more intense inflammation was recorded when certain oxidized sterol derivatives were used. Some of these oxysterols were also cytotoxic and injection of an oxidized sterol fraction has been shown to be angiotoxic, with resulting arterial thickening and fibrosis.[277] The presence of oxysterols in stored food stuffs and their possible presence over the surface of cholesterol crystals in experimental atherosclerosis may be related to the onset of necrosis in certain experimentally induced lesions.[278] In man, important accumulations of cholesterol and cholesterol crystals could not incite any particular reactions in the avascular intima and this absence of response to injuries contrasts with the magnitude of such changes in experimental models.

Whereas epidemiological studies have revealed many important risk factors for coronary heart disease, *the natural history of coronary atherosclerosis is studied experimentally by exaggerating a single risk factor*: *hypercholesterolemia*. It appears as one of the main risk factors for coronary heart disease in industrialized societies, but the conclusions one can draw only from cholesterol-induced lesions are limited. An illustrative example of these limits is that in man, important clinical manifestations of coronary heart disease, such as severe arrhythmias and sudden cardiac death cannot be clearly related to lipid metabolism disturbances.

The observations that mechanical and hemodynamic injuries can cause lesion development in human coronary arteries and the observations that repeated or continuous injury leads to local thrombosis, impart some urgency to the search for more appropriate animal models.[279] In addition, certain investigators believe that the time is ripe for passage from a subjective analysis of data obtained in experimental models, to one which is "totally objective", based on a computerized operator-interactive system for measurement of experimentally induced lesions.[138] This method, which utilizes a microcomputer, a high resolution digitizer tablet, dual floppy disc drives, and a videomonitor, is considered able to allow the investigator to detect subtle changes in the structure of lesions which might otherwise have gone unnoticed.

In spite of this considerable technical progress, an intriguing question still remains: are the basal arterial wall responses as known from the so-called experimental atherosclerosis applicable to man? Can some particular features of early atherosclerotic lesions of human coronary arteries be understood in terms of certain arterial wall changes obtained in laboratory animals? An adequate answer cannot be offered after more than seven decades of intense and uninterrupted effort.

B. The Rabbit Model

At the beginning of this century, Anitschkow and Chalatow[280] proposed the use of 0.5% crystalline cholesterol in highly unsaturated sunflower seed oil and rabbits as experimental models to produce atherosclerotic lesions. For many decades, the rabbit remained the unique laboratory animal for such research, and this model was the foundation of much investigation, involving feeding experiments of one kind of another, too numerous to mention. This model was considered by many pathologists, epidemiologists, clinicians, and particularly experimentalists to be a clear demonstration that hypercholesterolemia is both unnecessary and sufficient to produce atherosclerosis. Moreover, hypercholesterolemia was and continues to be considered the mechanisms which produces lesions, determines the progression of lesions, and operates in precipitating myocardial clinical manifestations. The passionate partisans of

the lipid theory of atherosclerosis rapidly reached the conclusion that the atherosclerotic lesions are no more than a pathologic reaction of the arterial wall to the invading cholesterol.

The size, availability, and cost of rabbits as a model are favorable for large-scale studies, as they are frequently used for the investigation of many aspects of atherogenesis, including the onset of lesions, vascular injury, arterial wall and particularly smooth muscle cell and endothelial cell metabolism, dietary and hormonal changes, platelet adhesion and aggregation, alteration in blood clotting mechanism, etc.

Rabbits are very susceptible to dietary-induced hypercholesterolemia and to the development of arterial lesions rich in lipids and particularly in cholesterol; an important relationship has been detected between dietary cholesterol and this form of experimental atherosclerosis.[281]

Upon cholesterol feeding, marked hypercholesterolemia occurs, followed by the onset of lipid-laden cells and of early lesions of fatty streak-like type which are different from those of spontaneous lesions predominate in the aortic arch and thoracic aorta and rarely involve the coronary and cerebral arteries. Therefore, the rabbit is not an adequate model for the study of the natural history of coronary atherosclerosis.

Other limitations are also worth mentioning: they are herbivores: cholesterol metabolism is peculiar: arterial lesions do not duplicate human atherosclerosis as concerns fibromuscular plaques, gelatinous lesions, intimal necrotic areas, incorporated thrombi and intramural thrombi — even fatty streak-like lesions have a different topography and are produced under conditions of extreme hypercholesterolemia. These experimentally induced lesions usually do not become complicated,[273] but some results could be obtained in the aorta of rabbits by feeding alternately normal and atherogenic diets for 2- to 3-month periods over a total period of up to 2 years.[281] Immunologic vascular injuries by means of repeated injection of foreign serum proteins have also been reported to give rise to complicated plaques in rabbits fed an atherogenic cholesterol-rich diet.[282]

A study of lipid inclusions in the experimental atherosclerosis of rabbits revealed that the three-dimensional configuration, crystallographic characteristics, physical properties, and chemical compositions are similar to those in human atherosclerosis.[106]

The simplest and possibly earliest lipid-containing lesion seems to be a subendothelial accumulation of unesterified cholesterol, detected with filipin dye.[283] It appears to precede morphologic changes in the endothelium and accumulation of subendothelial lipid-containing foam cells. Subendothelial accumulation of unesterified cholesterol may represent a stimulus for the migration of monocytes into the developing intima and a source of the cholesterol that accumulates within these cells. The possibility of taking free cholesterol directly as "extracellular liposomes" has recently been suggested.[284]

Studies aimed at the identification and origin of lipid-containing cells of the fatty streak-like lesions in experimental atherosclerosis of rabbits showed that they are mainly composed of lipid-laden macrophages. More advanced fatty streaks included a mixed population of lipid-laden macrophages and smooth muscle cells.[285] Populations of foam cells obtained from experimental diet-induced lesions showed that the majority of cells can be characterized as macrophages on the basis of several distinct markers and functional properties. These mononuclear macrophages in lesions might release elastase and collagenase and secrete factors affecting smooth muscle cell growth and functions. Such experimentally induced lesions very rarely progress to complicated ones and therefore in rabbit arteries, the presence of thick fibrosis, hemorrhage, ulceration, rupture, and thrombosis of plaques may be seen only by chance. These data refer to the aorta; in the coronary arteries the experimentally induced lesions usually develop in the small intramyocardial vessels.[286] In man, these small vessels are not involved by atherosclerotic lesions, but by nonatherosclerotic small vessel disease of inflammatory, allergic, immune, and genetic origin.

This absence of atherosclerotic lesions in the major coronary arteries of rabbits submitted

to cholesterol-rich diets contrasts with the accumulation of massive amounts of cholesterol in liver, spleen, lymph nodes, skin, and myocardium. In particular, the cells of the reticulo-endothelial system become massively engorged with cholesterol. This generalized lipoidosis has led to speculation about the significance of such experiments in relation to human coronary atherosclerosis. It is also becoming increasingly apparent that the rabbit's lipid and cholesterol metabolism substantially differ from that in human beings.

Many attempts have been made to augment the similarity of the rabbit lesions to human lesions. Thus, for instance, prolonged cholesterol feeding at a low level produced more fibrous and less cellular lesions.[287] Rabbits fed with diets which had fat and cholesterol levels similar to those consumed by human beings in North America showed a prevalence of proliferative lesions with fibromuscular caps and areas of necrosis. In these rabbits, serum cholesterol levels ranged from 138 to 532 mg/100 mℓ.[288] In addition, chronic immune complex damage, chronic carbon monoxide exposure, and a semisynthetic diet (containing cholesterol, butterfat, and sucrose) all were able to convert a lipid-rich lesion into a predominant proliferative one, rich in smooth muscle cells and exhibiting many characteristics of the advanced human atherosclerotic lesions. Such conversions were pioneered by Constantinides[281] 2 decades ago.

Starting from this new line of investigation, many investigators used a multitude of additional procedures which produced injuries to the artery wall of rabbits submitted to cholesterol-rich diets, in an effort to obtain more constantly and more rapidly advanced atherosclerotic lesions. Among these additional procedures, it might be of interest to remember:

— Mechanical trauma: scarification, insertion of wire coil, distension with a balloon catheter introduced into the lumen
— Air embolism
— Injection of particulate suspensions
— Various types of external compressions
— Vasotoxic agents (chemical injuries produced by means of epinephrine, norepinephrine, allylamine, aminopropionitrile, overdosage of vitamin D, alkaloids, etc.)
— Intravenous injection of blood clots
— Choline and vitamin B_6 deficiency
— Hormonal manipulation
— Disorder of reticulo-endothelial system function
— Freezing
— Electrical stimulation
— Bacterial infections
— Toxi-infectious factors
— Allergic inflammation
— Various hypoxic states
— Lesions caused by experimentally induced arterial hypertension
— Lesions caused by experimentally induced nervous system injuries
— Many other procedures too numerous to be mentioned

The results of these innumerable experimental procedures were considered cogent support that in rabbits arterial wall changes to various types of injuries are qualitatively uniform, regardless of the nature of the damage and that the process of repair would consist of the same succession of stages. Quantitatively, however, the reaction differs, depending upon the nature and intensity of the damaging factor and the duration of its action upon the arterial wall. According to this view, *injury to the arterial wall is probably a primary causative factor in atherogenesis*,[281] the resulting proliferation, inflammation and necrosis, and other features of repair favoring the deposition of lipoproteins at the site of injury and thereby

leading to the onset of atherosclerotic lesions. Repeated endothelial injury could be followed by the onset of lipid-rich lesions even in animals on a normal diet and such lesions did not form or were markedly inhibited in severely thrombocytopenic animals.[289]

Injuries to the arterial wall produce not only accelerated atherogenesis in susceptible vascular segments, but also the onset of atherosclerotic lesions in resistant vascular segments. Lipid-rich lesions appeared in only 3 weeks in injured rabbit vessels with an average terminal serum cholesterol as low as 145 mg/100 mℓ, while normal animals with an even five times greater terminal cholesterol level failed to develop lesions in a similar period of time. Many results published in the available literature demonstrate that injuries to the arterial wall lead to an accelerated onset of lipid-rich lesions in rabbits fed cholesterol-rich diets and that lipid deposition in arteries may occur at much lower serum cholesterol levels than in uninjured vessels within a given period of time. There is good reason to believe that in all foci of injury an inhibition of respiration and oxidative phosphorylation occurs, leading to a reduced production of energy-rich ATP bonds; these become inadequate for all energy-requiring processes, such as protein and enzyme synthesis. Likewise, a reduction of phospholipid synthesis was revealed, with subsequent diminished removal of accumulated lipids. A vicious circle develops that finally leads to a failure of all defense mechanisms of the artery wall against damaging agents of whatever origin.

In addition, particular forms of injuries are present; subtle injuries to the endothelium able to alter its primary function to act as a container of the blood and/or its function to form a thromboresistant barrier; to metabolize vasoactive substances; to act as a permeability barrier to plasma constituents; and to produce prostaglandins and many other functions. If desquamation occurs and the injury is chronic and is associated with hypercholesterolemia, conditions develop for the onset of lipid-rich lesions.

The wide use of many types of injuries to the arterial wall has created a peculiar situation in which atherosclerosis does not appear as a pathologic entity, or as a disease in the real sense of this term, but as a succession of various responses, or response patterns, of the artery wall to different damaging agents. These response patterns include: smooth muscle cell proliferation, elaboration of secretory products, lipid accumulation in cells and in intercellular areas, platelet adhesion and aggregation, thrombus formation, etc. Adhesion of monocytes is also a characteristic feature of such early reactions to injuries. Some of these monocytes may release growth factors and stimulate smooth muscle cell proliferation, others may give rise to macrophages and contribute to the intimal cell population and particularly to the onset of fatty streak-like lesions. These fatty streak-like lesions of rabbit arteries are considered by the great majority of investigators as being atherosclerotic in nature. However, it is not possible to overlook their usual absence of progression toward advanced forms with ulceration, rupture, and superimposed thrombus and their usual association with the presence of extraarterial lipid accumulations having the hallmark of a lipid-storage disease.[290]

C. The Rat, Bird, Dog, and Swine Models

The rat model of atherosclerosis has been neglected in recent years, largely due to the widespread belief that these animals are resistant to experimental disease. Evidence to the contrary was provided by Joris and Majno,[292] particularly as concerns the early stages of atherogenesis.

Feeding 40% peanut oil to rats which do usually not develop spontaneous atherosclerosis resulted in lipid-laden plaques in the aorta and in the proximal segment of the major coronary arteries.[293] If the diet included as fat 40% butter, the animals developed massive intracardiac thrombi and exhibited large areas of myocardial necrosis without stenotic or occlusive lesions in the coronary arteries. In a comparative study in which rabbits, guinea pigs, and rats received a high lipid diet containing coconut oil and cholesterol for 3 months, the less

pronounced changes appeared in rats; likewise, in rats the increase in blood serum lipids was smallest, with unchanged values of high density lipoproteins.[294]

Rats were also recommended as a particular model for atherosclerosis in order to analyze the fibroproliferative aspects of atherogenesis, based on the use of Lipofundin S, a soybean oil extract stabilized to a particle of chylomicron size. It produced enlargement of the subendothelial space, proliferation of smooth muscle cells, and accumulation of basement membrane-like material.[295]

In pigeons, aortic atherosclerosis at the celiac bifurcation may be evidenced in very young White Carneau birds, whereas coronary atherosclerosis does not develop until later in life. The lesions include many foam cells, encroach upon the lumen, and disrupt the internal elastic membrane, foam cell accumulation being surrounded by an important amount of fibrotic connective tissue.

White Carneau pigeons are one of the few animals models that develop severe atherosclerosis spontaneously, while consuming a cholesterol-free diet, usually a normal grain diet. They are particularly useful as a model for studying the natural history of atherosclerosis, since by age 4, nearly all birds have naturally occurring lesions.

The extent and severity of these lesions can be significantly increased and the timing of its occurrence accelerated by means of cholesterol-rich diets, atherosclerotic lesions including large foam cell accumulations being produced within 10 weeks. White Carneau pigeons provide an experimental model which allows comparison of naturally occurring lesions with nascent cholesterol-induced lesions. This investigation could start with the earliest changes induced by cholesterol-rich diets, such as a reduction in glycocalyx thickness as a prelesion change, associated with the appearance of endothelial cells with an atypical feature.[296] Such prelesions give rise to early lesions which can be followed up to the onset of complicated plaques; of note is the tendency of some plaques to ulcerate, rupture, and to show superimposed thrombi associated with plaque vascularization, plaque hemorrhage, and medial thinning.[297] Cholesterol-rich diets accelerate and aggravate the evolution of lesions toward complicated plaques and myocardial infarction can be considered a cause of death of some of the birds. According to some observations, myocardial ischemia and necrosis result from plaque emboli and not from plaque occlusion. Nevertheless, the White Carneau pigeon is of particular interest for all experimentalists involved in atherosclerosis research and is recommended as a suitable model to study the effects of experimental manipulations on the occurrence of plaque complications.

Other advantages of this experimental model: pigeons are inexpensive to keep, easily manipulated, reproduce readily and are long-lived (up to 20 years under laboratory conditions).

The presence of spontaneous atherosclerotic lesions only in certain species advocates for a genetic origin of this susceptibility to atherosclerosis, which does not seem related to sex and diet.[298] The offspring of White Carneau (intensely susceptible) and Show Racer (resistant) crosses have an intermediate level of susceptibility to atherosclerosis. It seems possible to select pigeon breeding pairs for the production of offspring with increased or decreased susceptibility to the onset of atherosclerotic lesions, as well as to myocardial infarction.[299]

Show Racer pigeons remain resistant to the development of atherosclerotic lesions even if they are submitted to cholesterol feeding. Upon cholesterol feeding, there is a marked increase in total cholesterol concentration in both breeds, but only White Carneau pigeons show a particular susceptibility to an accelerated onset and progression of atherosclerotic lesions. Since both breeds have a more or less similar lipoprotein pattern, this implies that susceptibility to the onset of atherosclerotic lesions is mainly related to the macromolecular constitution and organization of the arterial wall, as determined by genetic factors.[300,301]

A particular observation refers to the concentration of cholesteryl esters which were found to be significantly higher in the intima cushions of pigeons resistant to atherosclerosis. This would indicate that contrary to the general belief, cholesteryl esters are not atherogenic and

that additional factors are probably needed. In this respect, this observation is interesting: one of the earliest changes recorded in susceptible pigeons is the lesion area is the enhanced formation of prostaglandin E_2 (PGE_2) which has an inhibitory effect on cholesteryl ester hydrolase.[302]

Another important observation is that the arterial smooth muscle cells of White Carneau pigeons lack a functional LDL receptor pathway and in this way resemble cells from human beings with homozygous familial hypercholesterolemia.[303] As a result of the lack of a functional LDL receptor pathway, pigeon arterial smooth muscle cells do not maintain cholesterol homeostasis through the controlled uptake of exogenous LDL-cholesterol as in humans, in which this homeostasis is usually less influenced by cholesterol synthesis of efflux. In pigeons, the smooth muscle cells of the large and medium-sized arteries are able to synthesize cholesterol at a rate of up to 100 times greater than in mammalian cells, accumulation of excess cholesterol being prevented by rapid efflux of endogenously synthesized cholesterol.[304] The difference in susceptibility to atherosclerosis between the above-mentioned two pigeon breeds is not due to the presence of an LDL-receptor pathway in one breed and its absence in the other. The onset of spontaneous atherosclerosis and its aggravation by cholesterol-rich diets do not require a functional LDL-receptor pathway because the lesions appear even at relatively low serum LDL-concentrations.

Many other studies have been conducted in an effort to identify the metabolic parameters related to the particular susceptibility to both spontaneous and experimental atherosclerosis of White Carneau pigeons. A prevalence of glycolysis as the main source of energy, a more important synthesis of proteoglycans and prostaglandins, and an increased ratio of cholesterol esterase synthesizing to hydrolyzing activity were revealed. Accelerated glycolysis present in atherosclerosis-susceptible pigeons is believed to predispose to lesion development.[305] The mitochondria of the susceptible White Carneau pigeons showed a deficiency of the glycerol-3-phosphate shuttle with significant increases in the steady-state levels of both arterial wall lactic acid and glycerol phosphate. In addition, a lack of regulation of transhydrogenase to ATP was recorded, leading to augmentation of the mitochondrial level of the NADPH/NADP ratio, which could reflect an increased synthesis of lipids.[306] A significant difference was also revealed in post-translational processing of proteoglycans in the two pigeon types which may influence their susceptibility or resistance to atherosclerosis.[307] An increased content of dermatan sulfate in the progressing atherosclerotic plaque of the White Carneau pigeon was recorded which may suggest an augmented capacity to bind LDL macromolecules.[308]

The genetic predisposition to atherosclerosis of White Carneau pigeons in terms of quantitative and qualitative differences at the level of the arterial wall is of considerable interest since there are no differences in risk factors between atherosclerosis-susceptible and atherosclerosis-resistant pigeons. An eloquent example is offered by the investigations centered on endothelial changes on the existence of subtle endothelial injuries, which are not necessarily followed by denudation at the site of lesion formation. These subtle alterations may include increased permeability to plasma proteins (fibrinogen, albumin), lipoproteins (cholesterol), and alterations of the luminal glycocalyx.[309,310] These alterations could be detected in the atherosclerosis-susceptible areas but not in the nonsusceptible. It is tempting to presume that endothelial cells which cover the arterial wall areas prone to atherosclerotic involvement have a different behavior in the presence of injuries produced by hemodynamic stresses than endothelial cells lining nonsusceptible regions. In addition, monocytes preferentially adhere to areas where the endothelium is injured and in the cholesterol-fed birds they may become lipid-filled cells. Both endothelial alterations and leukocyte adherence are early events in naturally and experimentally induced atherosclerotic lesions, and occur prior to the appearance of visible lesions. In a subsequent stage, focal nodules of smooth muscle cell proliferation can be seen which expand laterally with time.[311]

Atherosclerosis-susceptible White Carneau pigeons were also compared with atherosclerosis-resistant Show Racer pigeons in terms of biotransformation and bioactivation of carcinogenic chemicals.[312] The results document marked differences in biotransformation and bioactivation of carcinogenic hydrocarbons which reflect metabolic differences similar to those observed in carcinogenesis-susceptible and -resistant strains of mice. Owing to these metabolic differences one can suggest that some hydrocarbons might act as atherogenic agents.[312]

Chickens also develop naturally occurring lesions, but atherosclerotic involvement is not as consistent as in pigeons and the model requires additional dietary manipulations. The lesions are lipid-rich and calcium deposition within lesions is common, but the birds do not die of complications of the plaques. Of particular interest is the finding of more severe atherosclerotic involvement detected in chickens with Marek's disease herpesvirus.[313] An atherogenic diet enhanced this virus-related atherosclerotic involvement and increased the number of fatty-rich lesions, as compared to virus-infected birds fed a normal diet, in which the prevalent lesions occurred as fibromuscular plaques.

In turkeys, arterial hypertension is probably the most potent atherogenic factor. Some breeds of turkeys may die of dissecting aneurysm induced by atherosclerotic lesions.

In the dog, almost all experiments have included both cholesterol feeding and suppression of the thyroid function in order to produce atherosclerotic lesions. Many months of cholesterol feeding and thyroid suppression are necessary for the onset of such lesions, the number of fatty filled cells correlating closely with serum cholesterol levels and the presence and length of hypercholesterolemia. Consequently, the animals with the highest level of serum cholesterol over the longest period of time are those which show the most extensive and severe atherosclerotic involvement. A particular feature of the dog model of atherosclerosis is the occurrence of lesions in small caliber arteries before major coronary arteries are involved. Additional particularities are the absence of a thickened intima before the development of lesions and the prevalent involvement of the media. The lesion most frequently encountered in the arteries of the dog is lipid infiltration in the middle third of the media, which shows large aggregations of foam cells, associated with distinct hypercellularity.[285] The advanced lesions do not develop a thick fibrous cap, but show cellular degeneration, formation of extracellular cholesterol crystals, hemorrhage, dystrophic calcification, ulceration, and thrombosis.[314] Intimal changes develop secondarily to this medial lesion.

The swine model is of particular interest for the study of the natural history of atherosclerosis, some investigators assuming that "pigs are nearly ideal as animal models of human atherosclerosis".[273] These animals develop atherosclerosis naturally, without dietary manipulation and when diet is supplemented with cholesterol, the onset and progression of atherosclerotic lesions is accelerated. Spontaneous atherosclerosis is first seen at about 6 months of age as fibromuscular plaques which occur as nodular proliferations within the thickened intima. Some of these nodular proliferations may acquire the feature of elastic hyperplastic nodules, others of lipid-rich nodules. Of particular interest are the results of studies demonstrating that the lesions arise from intimal cell masses which are present in specific anatomical sites from birth.[315] Since coronary human atherosclerotic plaques frequently develop on their own from the thickened intima,[316] this experimental model seems to represent an adequate basis to analyze atherogenesis in a different way than starting from the supposition of fatty streak-fibrous plaque conversion.

The severity of atherosclerotic lesions produced in swine increases with age and some become complicated plaques with hemorrhage, ulceration, and thrombosis. All these severe plaques may develop at serum cholesterol levels around 100 mg/100 mℓ or even less. When cholesterol is supplemented to the diet and the cholesterol level augments up to 1000 mg/100 mℓ, all fibromuscular plaques appear as lipid-rich lesions with a large necrotic center, including many cholesterol clefts. Lesions increase in extent and severity with duration of

cholesterol feeding (cell death occurring in all plaques), but plaque ulceration, rupture, and thrombosis are very rare events. To obtain complicated lesions, some experimentalists have combined hypercholesterolemia with injuries. A particular type of injury was obtained following irradiation of the thorax of the pig coupled with cholesterol feeding; this may lead within a few months to myocardial damage. A combination of atherogenic diet and mechanical injury was followed by the onset of three distinct types of arterial lesions: (1) nonraised lesions composed mainly of lipid-laden foam cells, (2) raised lesions including both foam cells and spindle cells, and (3) complicated plaques with a necrotic center and a fibrous cap.[317]

In certain studies a strong correlation appeared between the level of serum intermediate density lipoproteins (IDL) and the susceptibility to atherosclerotic involvement. Consequently, this level was used to predict whether a given swine would be a high or a low responder to atherogenic diets. IDL total values in milligrams per decaliters ranged from 33 to 66 in the low proliferative responder group and from 117 to 518 in the high responder group.[318] IDL-cholesterol constituted only 6% of the total cholesterol in the low-proliferative responder group of 20% in the high responders. In swine, it is thus possible to obtain a high proliferative response to hyperlipidemic diets with high IDL values, regardless of the levels of LDL. In vitro studies revealed that several lipoprotein components appear to have mitogenic activity, the highest VLDL (very low density lipoproteins), followed by LDL (which includes IDL), with HDL (high density lipoproteins) last.[319]

Pigs fed on a diet high in both fat and cholesterol were largely used to demonstrate the complex role of blood monocytes in experimental atherogenesis. In pigs fed atherogenic diets, many areas of increased endothelial permeability to protein (as revealed by the uptake of Evans blue dye) and increased numbers of monocytes adherent to the endothelium were seen. Monocytes and lymphocytes occur in the subendothelium as normal constituents of the swine arterial intima.[320] Most of these cells are associated not only with the onset of atherosclerotic lesions, but also with changes in lipid metabolism, immunologic reactions, and nonspecific inflammation. There are also unclassifiable or primitive cells of myogenic origin, intermingled with monocytes and lymphocytes. It is tempting to presume that many subendothelial elements are modulation forms of smooth muscle cells.[321] Accumulation of these cells was detected both before and during the onset of fatty streak-like lesions; in addition, these cells may be plausible candidates for removing lipid from intimal lesions.[322,323] *This relates the onset of fatty streaks to a monocyte clearance system*, the ratio of invading monocytes to emerging lipid-filled elements decreasing in the course of fatty streak development until a 1:1 ratio is achieved. When this ratio occurs, the fatty streak is fully developed and the lesion does not progress further. This progression may be determined by a failure of the monocyte clearance system reflected by the incapacity of some lipid-laden foam cells to migrate back into the bloodstream, by crossing the arterial endothelium. The invasion of monocytes, the endothelial damage which may result, the further accumulation of lipid, and the absence of foam cell egress all contribute to the development of more advanced atherosclerotic lesions. Additional contributory factors are growth substances derived from macrophages and platelets.

Prostaglandin metabolism seems to be strongly related to swine atherogenesis, inhibition of lesion development being produced through changes in prostaglandin metabolism with no relation to changes in plasma lipids.[324]

Minipigs are also considered able to develop atherosclerotic lesions of a human type and some of these lesions may progress to complicated plaques. Diet-induced lesions are associated with a rise in all types of lipoproteins, except HDL and especially HDL_2, which decrease, this model being considered adequate to analyze lipoprotein changes in response to high cholesterol intake.[325] A morphometric method has been proposed to express the topographic distribution of atherosclerotic lesions in unambiguous statistical terms.[326] Com-

puter-stored images of opened sudan IV stained coronary arteries from hypercholesterolemic minipigs revealed many areas of high probability for the onset of lesions. Areas of high probability are associated with the entrance regions and the lateral leading edges of the major flow dividers. The maps obtained show that sudanophilic lesions are localized in a characteristic topographic distribution and that the regions immediately distal to the large branch points are shown to be areas of low probability. A particular observation refers to the topography of spontaneous atherosclerotic lesions of old animals which seems to be similar to the topography of diet-induced lesions in younger minipigs.[325,326]

Also worthy of note is the occurrence of spasm provoked by intracoronary of intravenous administration of histamine in the coronary arteries of swine. Spasms mainly develop in the denued areas of the left circumflex artery *endothelial balloon denudation* where atherosclerotic changes also appear predominant. This experimental model may be considered adequate for analyzing the relationship between coronary spasm and coronary atherosclerosis.[327]

D. The Monkey Model

In the 1950s and 1960s, nonhuman primates became the preferred animal model for atherosclerosis research, especially in the U.S. Many investigators and especially many experimentalists consider that nonhuman primates provide the best model for the studies of factors and mechanisms involved in atherogenesis. Not only are the nonhuman primates phylogenetically closer to man than other animals, but they have also been demonstrated to present many anatomic, physiological, biochemical, and metabolic similarities to human primates. Thus, for instance, the coronary anatomy of certain macaques resembles that of man more closely than does the dog, as regards the origin of the posterior descending artery, supply of the sinus node and atrioventricular node, and perfusion of the interventricular septum.[328] In addition, the left anterior descending artery was often found to course under muscular bridges for varying distances. There are thus reasons to believe that nonhuman primate species represent remarkable prototypes of the anatomy, physiology, pathophysiology, and pathology of human coronary arteries; consequently, there are also reasons to believe that experimental atherosclerosis in nonhuman primates is rather similar to human disease.[329]

Some experimentalists insist on the particular advantage offered by the macaque model to produce lesions with small quantities of cholesterol in the diet. The average cholesterol may be 300 mg/100 mℓ, similar to that in the average American diet, or may also be 200 mg/100 mℓ, as detected in subjects ingesting a prudent diet. The lesions develop at both blood cholesterol levels, but in monkeys fed the average American diet, these lesions were about three times more frequent and severe than in animals fed the prudent diet.[330] Even small quantities of dietary cholesterol that failed to elevate serum cholesterol level were able to produce both intimal thickening and intimal sudanophilia.[331] On the other hand, when the amount of cholesterol in the diet was high, not only elevated cholesterol values resulted, but also cholesterol accumulation in the liver, spleen, heart, skeletal muscle, fat, and skin.[332,333] This suggests that this last form of experimental atherosclerosis may be more closely related to human familiar hypercholesterolemia than to human atherosclerosis.

In addition to hypercholesterolemia, nonhuman primates seem to be adequate models for the study of other major risk factors for coronary heart disease. The response to cholesterol feeding is strongly influenced in some nonhuman primates by age, sex, diet composition, genetic factors, arterial hypertension, endocrine abnormalities, and associated diseases. The extent and severity of diet-induced atherosclerosis depend heavily upon genetic background, reflected by the existence of hyper- and hyporesponders to cholesterol-rich diets. Experimentalists can dissect the influence of each major risk factor on the onset and progression of coronary atherosclerosis, and can try to translate this influence into measurable parameters at the coronary artery wall level.

A major difficulty in the study of the natural history of coronary atherosclerosis with the aid of the monkey model is the rarity of obstructive lesions. In spite of the fact that the arteries of monkeys given atherogenic diets develop marked intimal thickening associated with the onset of plaques, luminal size apparently changes minimally.[334] In addition, focal atrophy of the media occurs as a common finding in primate experimental atherosclerosis. From a hemodynamic point of view, total vascular resistance is increased, lumen size is not decreased, and vasoconstriction responses are not altered.

The particular importance of the monkey model considerably increased when it was found that in a large colony of monkeys undergoing progressive experimental atherosclerosis, the rate of myocardial clinical manifestations might be similar to that which would be expected to occur in human subjects with advanced atherosclerosis. This demonstrated that nonhuman primates as a group are also suited for investigation of the natural history of coronary atherosclerosis. Selective breeding programs are now underway whose aim is to produce a population of nonhuman primates in which those characteristics important in human atherogenesis are enhanced and those not found in the human are repressed.[273]

Some monkeys develop spontaneous atherosclerosis, particularly rhesus monkeys, without any evidence of elevation in either blood or arterial wall lipid levels. The lesions show a prevalent fibrous character and may be called fibrosclerotic or fibrohyaline plaques, occurring as elevated focal proliferations in a diffuse fibrosclerotic intimal thickening. Feeding rhesus monkeys on a ration closely resembling the average American diet (in relation to basic ingredients, method of preparation, and composition) led to the onset of coronary lesions which resembled in many ways those observed in young American males.[335] Many of the lesions observed in the average American diet-fed monkeys resembles those considered as gelatinous plaques.[336] These lesions were obtained with a blood cholesterol level not very different from that found in the American adult population. If a prudent diet was used which was low in saturated fat and cholesterol, the blood cholesterol level remained below 200 mg/100 mℓ and the lesions did not appear over a 2-year period.[337]

All male rhesus monkeys receiving low cholesterol food and having a blood cholesterol mean value of 142 mg/100 mℓ had intimal thickening, occurring either as branch pads or cushions, or as diffuse thickening, the highest degree of intimal thickening being recorded in the cushion at the apex of the bifurcation of the left main coronary artery.[338] When the high-cholesterol diet was given, the mean value of blood cholesterol rose to 400 mg/100 mℓ and then to 575 mg/100 mℓ; this was followed by the occurrence of lipid inclusions in some smooth muscle cells of the preexisting branch pads or cushions, associated with a moderate increase in the thickness of intimal cushions and adjacent intima, as a result of the accumulation of foam cells, macrophages, and debris. Forty weeks after starting the high cholesterol diet, extensive atherosclerotic lesions blended with the preexisting intimal cushions, the largest lesions containing several layers of foam cells adjacent to the lumen. On the other hand, cell debris and extracellular lipid tended to accumulate in pools adjacent to the internal elastic membrane.[338] The above results show that the macaques offer some of the best experimental models of the natural history of coronary atherosclerosis, since in children aged 6 to 15 years, the first atherosclerotic plaques were seen to develop in preexisting branch pads or cushions.[339]

Before starting any experimental procedure in monkeys, it is necessary to establish a baseline of naturally occurring atherosclerotic lesions. In addition to the possible presence of spontaneous atherosclerotic lesions, a disadvantage of the monkey model is that the animal is expensive, hard to handle, and uniform atherosclerotic involvement in adults is difficult to obtain. The conduct of the studies requires excellent facilities and expert husbandry and carries a certain element of risk.

Whereas the baboon submitted to cholesterol-rich diets develops only fatty streaks, the rhesus monkey shows extensive and advanced atherosclerotic lesions. These differences

demonstrate that various nonhuman primate species may show various degrees of susceptibility to diet-induced atherosclerosis.

In macaques, the high-lipid diets produce an intense intimal proliferation, followed by necrosis and the conversion of lipid-rich lesions into typical advanced lesions, particularly in animals receiving diets in which thiouracil and sodium cholate are used as adjuvants.[340] Any proliferative lesions that reached 0.85 mm will become necrotic unless it becomes vascularized. The thickness of the proliferative lesions was not the only factor determining whether an intimal fat accumulation will become necrotic. Toxic products resulting either directly or from diet may predispose to or cause intimal necrosis. The rhesus monkey fed a mixed natural diet enriched with cholesterol and butter consistently develops not only advanced plaques, but also myocardial infarction associated with the presence of stenotic plaques in the major coronary arteries.[341,342] These advanced plaques may show calcification and thrombosis and certain experimentalists were impressed by the similarity of such advanced lesions to those frequently encountered in human coronary arteries.

Diet-induced atherosclerosis in rhesus monkeys *(Macaca mulata)* or cynomolgus monkeys *(Macaca fascicularia)* has a remarkable similarity in both anatomic location and morphologic appearance. However, at equal or lower blood cholesterol levels, cynomolgus monkeys develop more severe lesions than do rhesus monkeys. These lesions contain accumulation of apoprotein B in amounts similar to those found in man. Based on this similarity it is tempting to assume that diet-induced hypercholesterolemia in such animals may be a valid model for studying the role of LDL in atherogenesis.[343] The lesions of cynomolgus monkeys more frequently involve both intima and media, with greater fibrous response, more cholesterol clefts, and extracellular lipid than the lesions of rhesus monkeys. The difference in atherosclerotic involvement between these two macaques may simply reflect a greater blood cholesterol response of cynomolgus monkeys to dietary cholesterol and, as a result, a more abnormal spectrum of lipoproteins, particularly of the β-class. Cynomolgus monkeys fed a diet containing cottonseed oil and added cholesterol as a supplement to Purina Monkey Chow®, develop not only advanced atherosclerotic plaques in the proximal segment of the major coronary arteries, but also myocardial lesions that may produce sudden cardiac death.[344,345] Sudden death occurred after the animal has been on atherogenic rations for 12 months. Cynomolgus monkeys may appear not only unusually susceptible to the development of atherosclerosis, but the severity of lesions was also significantly higher, as reflected by an atherosclerotic index, among males compared to females fed the same atherogenic diet.[346] Like human females, the female cynomolgus macaques tend to have higher HDL levels than their male counterparts; on the other hand, male cynomolgus monkeys had larger LDL particles than females of similar age, containing more cholesteryl ester. A high correlation was revealed in some cynomolgus macaques between LDL particle size, HDL concentration, and the severity of coronary atherosclerosis.

Peculiar differences between rhesus monkeys and cynomolgus monkeys also reside in the fact that over 80% of cynomolgus monkeys had definite evidence of circulating Ag-Ab complexes, while rhesus monkeys did not and about 90% of rhesus coronary artery lesions were eccentric and intimal, while 75% of the cynomolgus lesions developing in the same length of time were concentric and transmural.[347]

Some macaques called pigtail monkeys *(Macaca nemestrina)*, following 3 to 4 months of hypercholesterolemia comparable to that found in human familial hypercholesterolemia (500 to 1000 mg/100 mℓ) showed only fatty streaks visible on gross inspection. Contrary to human fatty streaks, they were preferentially developed at branch sites and areas of bifurcations.[348] In these subendothelial foam cell accumulations, macrophages act as scavengers and tend to clear the subendothelium in which LDLs modified by the endothelial cells are engulfed by macrophages. Accumulation of lipoproteins may convert some macrophages into foam cells and the number of intimal macrophages and foam cells may augment

to the point where fatty streaks become visible to the naked eye. The increasing tension on endothelial cell junctions and marked thinning of the endothelial cells induced by enlarging lipid-laden macrophages, or the increased susceptibility of endothelial cells to shear forces and eddy currents at particular anatomic sites could lead to separation of endothelial cells, exposure of foam cells, and their eventual release into the circulation. The endothelium could be also damaged by oxidation products released by fatty-filled cells and by hydroxyl and free radicals, together with neutral and acid proteolytic enzymes. Such damages may convert a change which occurs as part of a protective inflammatory response into a lesion acquiring the features of an atherosclerotic plaque. Continued hypercholesterolemia seems to be required for monocyte attachment to the endothelium, enlarging the lesion which becomes able to progress to more advanced forms.[349] If the cholesterol level increased much more rapidly, the advanced lesions would be more rapidly observed. Platelet adherence, aggregation, and release of factors able to produce smooth muscle cell proliferation may also be involved.

African green monkeys develop atherosclerotic plaques that are more fibrous than those found in rhesus monkeys. Hypercholesterolemia per se was not directly responsible for the greater stimulation of collagen synthesis in African green monkeys than in rhesus monkeys, the difference recorded being associated with different genetic potentials.[350] Likewise, different genetic potentials could be detected as concerns the synthesis of heparan, dermatan, and chondroitin sulfates, the ratio of heparan sulfate to chondroitin sulfates plus dermatan sulfate being associated with increased susceptibility to atherosclerosis (high ratio) or with reduced susceptibility (low ratio).[351]

In stumptailed macaques, severe coronary atherosclerosis could be produced experimentally by means of cholesterol-rich diets, with the presence of lesions narrowing the lumen of all major coronary arteries and with a diffuse atherosclerotic involvement similar to that detected in patients with fatal myocardial infarction.

In the squirrel monkeys *(Samiri sciureus),* elevated blood cholesterol levels and intensification of atherosclerotic involvement were produced by means of repeated psychological stresses.[352] An intensification of atherosclerotic involvement could be also obtained after exposure to carbon monoxide, prolonged hypothyroidism, and particularly arterial hypertension. The coronary atherosclerotic lesions in the hypertensive group were about three times as largely developed as they were in the normotensive group.[353,354] There are few papers which demonstrate that hypertensive monkeys develop both intimal thickening and lesions similar to the "pearly plaques" frequently seen in human autopsy material. The monkey model represents one of the early pieces of evidence that arterial hypertension produces coronary intimal thickening and increases the plaque size.[355]

Many similarities were also detected between the natural history of coronary atherosclerosis in man and chimpanzees (*Pan*). The range of blood cholesterol concentrations in chimpanzees in 220 to 240 mg/100 mℓ, resembling the findings in Western societies. Newborn chimpanzees show coronary intimal thickening similar to that in human beings. Spontaneous coronary atherosclerosis is more severe in chimpanzees than in other species, the plaque showing a more abundant musculoelastic proliferation with ulterior lipid deposition. Both focal proliferation and lipid accumulation lead to vessel occlusion and focal myocardial necrosis and even pure myocardial infarctions were repeatedly observed. As is true with all studies of coronary atherosclerosis in nonhuman primates, there is a considerable individual variability in the extent and severity of lesions. After 15 to 20 months of atherosclerosis induction, some animals show only fatty streaks or very small plaques, others severe fibrofatty lesions containing both intra- and extracellular lipid, necrosis, cholesterol clefts, mineralization, hemorrhage, and a thick fibrous cap.

In essence, *dietary cholesterol is generally used as a sine qua non* for the induction of coronary atherosclerosis in nonhuman primates and marked hypercholesterolemia seems to be necessary for significant lesion formation in most, but not all species.

To induce experimental coronary atherosclerosis of adequate magnitude, dietary cholesterol must apparently exceed ordinary human intake by a considerable amount and hypercholesterolemia must be maintained at an average level found in human homozygous familial hypercholesterolemia, for a period of many months to several years.

The elevation of blood cholesterol levels after cholesterol-rich diets is primarily caused by increase in LDL; in some nonhuman primates this was associated with a decrease in HDL.

An important factor of variability seems to be the type of the fat included in the diet since peanut oil causes more fibroplasia, butterfat produces lesions rich in lipid deposits, coconut oil induces elevated lesions with minimal fibrosis, and corn oil gives rise to very few lesions. The atherogenic effect seems to be broadly associated with the degree of saturation of the fat and the induced rise in the blood cholesterol level. Some fats appear to bear a specific influence on certain selective aspects of lesion development and part of this influence seems to be distinct from the degree of hypercholesterolemia. In nonhuman primates, as well as in other experimental models, lesion morphogenesis and some special features of lesions appear partly related to specific fat combined with dietary cholesterol and partly related to resultant change in blood lipoprotein concentration and composition. Hypercholesterolemia remains the major focus of attention in the monkey model of atherosclerosis, but it appears to be only one of the major risk factors in the natural history of human coronary atherosclerosis.

E. Experimental Coronary Occlusions

Coronary occlusion represents an advanced stage in the natural history of coronary atherosclerosis, a stage in which myocardial clinical manifestations may occur as angina pectoris, myocardial infarction, or sudden cardiac death. The animal models based on cholesterol-rich diets and subsequent hypercholesterolemia are not adequate to produce such occlusions and myocardial ischemia, since the lesions rarely progress to advanced forms. Only in pigeons, pigs, and certain nonhuman primates may such obstructive lesions accidentally appear following prolonged cholesterol-rich diets and these are anecdotal aspects.

The absence of a standardized method able to give rise to coronary occlusive plaques by means of cholesterol-rich diets also explains the unexpected proliferation of such models. This attests to the interest in and need for an animal that will permit follow-up of the successive coronary changes which lead to lumen occlusion and to myocardial ischemia and necrosis. Since such models are not yet available, the experimentalists are obliged to use ligation of a major coronary artery, usually a normal vessel and to produce myocardial ischemia and necrosis in the absence of cholesterol-rich diets and of atherosclerotic involvement of the coronary arteries.

The most widely used method to obtain coronary occlusion is sudden ligation of the normal vessel in open chest and anesthesized animals. The method offers the possibility of exact timing of the onset and duration of blood flow cessation and implicitly of myocardial ischemia; releasing the ligature or clamp makes it possible to investigate the effects of myocardial revascularization.

Differences in the experimental models used refer to the species, state of sedation of anesthesia, open or closed chest preparation, extent of myocardium rendered ischemic, duration of ischemia, time of intervention, and many other parameters. Using coronary ligation in the conscious animal, some experimentalists consider that they avoid the suppression of cardiovascular reflexes and the frequent negative inotropic effect of anesthesia. Correct observations obtained from one model or set of models may not be confirmed by other models and particularly species differences must first be taken into consideration. Thus, for instance, in the pig occlusion of the right coronary artery produces infarcts of the posterior wall of the left ventricle and interventricular septum but not of the right ventricle.[356] The very poorly developed collateral circulation almost invariably leads to transmural infarction,

whereas in other species with well-developed collateral vessels, only subendocardial infarction or the absence of myocardial necrosis can be seen after a similar occlusion. In the cat, coronary occlusion usually results in rhythm disturbances, particularly bradycardia.[357] On the other hand, in sheep, slow stenosis of the coronary arteries led to death from heart failure after a few days since this animal lacks coronary collateral vessels and large transmural infarctions occur rapidly.[358] The baboon heart easily goes into fibrillation after coronary occlusion and therefore is not suitable for testing myocardial protective procedures. In fact, all animals which lack collateral vessels are not suitable for testing myocardial protective procedures since a myocardial region devoid of coronary supply after occlusion becomes inaccessible to drugs.[359] The poor development of collateral vessels in the baboon may also explain why, in spite of a similar duration of left anterior descending artery occlusion in baboons and dogs, infarct size appeared greater in baboons than in dogs.[360] Moreover, in the baboon transmural myocardial necrosis can be easily produced, whereas in dogs subendocardial necrosis prevails.

An interesting observation is also that in dogs coronary collateral flow exhibited a strong negative correlation with all forms of arrhythmias.[361] The lowest collateral flow ranges were almost invariably associated with ventricular tachycardia and fibrillation. As collateral function improved, the incidence of these arrhythmias decreased rapidly. A higher collateral flow was able to prevent arrhythmias even in the presence of large ischemic regions.

The relative well-developed network of collateral vessels makes the canine heart suitable for studies of experimental infarction and the results obtained might be partly transferable to man. Since the canine heart is provided with a well-developed collateral circulation after chronic coronary occlusion, it offers the possibility of influencing the severity of myocardial ischemia by pharmacologic means. The dog is also considered an adequate experimental animal to allow for accurate measurement of collateral flow and a standardized induction of collateral vessels and flow measurements was proposed.[362] The role of these vessels to maintain myocardial integrity seems to be considerable, since in the canine heart occlusion of the left anterior descending artery was followed by an ischemic area limited to 60% of the perfusion region of the respective artery, whereas in the pig 100% of the region supplied by the occluded left anterior descending artery became necrotic.[363] The collateral circulation which develops in dogs after chronic occlusion of a major coronary artery may protect the myocardium, but its functional capacity seems to be limited to resting conditions and it may become inadequate under physical stress.[364]

Likewise, the major biologic changes occurring during myocardial ischemia produced by sudden or chronic occlusion of a major coronary artery have been more deeply investigated in the canine model.[365] The possibility of exactly timing the onset and duration of ischemia and the complete cessation of antegrade flow are attractive features of this model, as well as the possibility of releasing the ligature or clamp which makes it feasible to study myocardial reperfusion. Important progress resulted from the use of ameroid constrictors: they consist of plastic material encased in a stainless steel ring that is slipped onto the artery. The ameroid plastic is a hygroscopic material that swells with the absorption of tissue fluids and results in progressive stenosis, followed by total occlusion after 2 to 3 weeks.[366] The tracer microsphere technique has also been employed to investigate the changes in collateral resistance after chronic coronary occlusion and the functional capacity of these vessels under conditions of cardiac stress.[364]

In essence, dogs were selected by many experimentalists as an adequate animal model for the study of the end stages of the natural history of coronary atherosclerosis and particularly of myocardial infarction, because lesions develop in the presence of some residual coronary perfusion via subepicardial collateral vessels. As in human beings, the location of these enlarged collateral vessels is related to the topography of the occluded artery and the anatomical branching pattern of the coronary arteries. In dogs, as in humans, the suben-

docardial zone is more susceptible than the subepicardial zone to infarction.[367] Finally, in dogs, as in man, three major sources of variation in infarct size were detected related to the anatomy of the area at risk, the adequacy of the collateral circulation, and the rate-pressure product.

Dogs are also considered adequate for the study of the effects of partial as compared to complete thrombotic occlusion of the left anterior descending artery. A complete occlusive thrombus was associated with full-thickness myocardial infarction, while a mural thrombus was associated with focal areas of myocardial injury, mainly subendocardial in location.[368] Certain models are based on the intravascular injection of substances able to produce multiple platelet aggregates and small thrombi which may obstruct small coronary arteries and their intramyocardial branches.

Important experimental studies were also carried out on canine hearts to demonstrate whether a particular therapy could limit experimental infarct size.[369] Attempts were also made to accurately define the region at risk to infarction, since it may allow the quantitatively assessment of the effect of therapeutic interventions. A clear superposition between the presumed region at risk and the perfusion bed of an occluded artery was rarely recorded since collateral blood flow may strongly influence delineation of the perfusion bed. The only constant results are those which demonstrate that a considerably smaller volume of necrotic myocardial tissue occurs with gradual occlusion as compared with rapid, sudden occlusion. The size of myocardial damage could be limited because collaterals are given sufficient time to provide blood flow to the impaired vascular bed during occurrence of the gradual occlusion. Techniques designed to alter this rate of development of coronary collateral vessels and to permit follow-up of the results of a pure slow occlusion of a major coronary artery have been reported.[370-372]

In addition to ligation, the techniques employed for the production of the end stages of the natural history of coronary atherosclerosis include the injection of necrotizing agents, direct or indirect embolization with sized microspheres, and various other types of arterial injuries. In addition, scintigraphic techniques have been developed which allow direct visualization of a myocardial infarct as a result of selective accumulation of certain radionuclides in the necrotic area. Myocardial imaging with radionuclides, associated with analysis of serial changes in enzyme activity, analysis of electrophysiological phenomena, electrocardiographic mapping technique, and post-mortem examinations may give a complex picture of the mechanisms involved and of the succession of changes which lead to the end stages of the natural history of coronary atherosclerosis.

Canine models were also used to detect hemodynamic factors which might be encountered in the clinical setting of cardiac arrhythmias. Since the pioneering work by Erlager in the early 1900s, numerous experimental models have been developed in an attempt to produce various types of blocks and particularly, complete atrioventricular conduction block. Two types of ventricular arrhythmias have focused the experimentalist's attention: those produced by the first ischemic event and those associated with the relief of ischemia or the so-called reperfusion arrhythmias. The cat was also used to investigate the relationship between myocardial infarction and ventricular arrhythmias, particularly after slow ligation of the left anterior descending artery. The cat seems to be particularly suited for the study of ventricular rhythm disorders and especially ventricular fibrillation which precedes sudden cardiac death. On the other hand, the dog seems to be a more adequate model than the cat for investigating sudden cardiac death precipitated by dissolution of an platelet plug or resolution of coronary vasospasm, either of which could induce ventricular fibrillation by perfusion-reperfusion mechanisms. Likewise, this model was largely employed to assess the effectiveness of many antiarrhythmic drugs in the prophylaxis of sudden cardiac death. Attempts have been made to relate the probability of ventricular fibrillation to small and large amounts of myocardium at risk.[373] A method has been devised for the production of complete atrioventricular heart

block in anesthesized closed-chest animals and this may open new horizons for a more complex study of the natural history of coronary atherosclerosis by means of experimental models.[374]

A major disadvantage of all experimental models based on acute occlusion is that a previously healthy myocardium abruptly becomes ischemic, not allowing any previous adaptation to ischemia. Also of note is the fact that the unoccluded segments of the coronary arteries of the experimental model are normal, whereas in man they are diffusely involved by atherosclerotic lesions in middle-aged subjects and elderly people. With the exception of an important hemorrhage in an atherosclerotic plaque, sudden occlusion of a coronary artery in man, equivalent to that produced by a silk ligature in a dog, probably occurs rarely, if at all. The natural history of human coronary atherosclerosis is characterized by the gradual accretion of small thrombi over a stenotic plaque on which an occluding thrombus may develop. This gradual stenosis usually develops in man over a period of time that allows preexisting collaterals to enlarge and compensate partially or completely the diminished blood flow to the myocardium of the impaired vascular bed. Thus, in man, gradual occlusion may result in absence or considerable reduction of myocardial ischemia and necrosis, compared to acute coronary occlusion which consistently produces myocardial infarction.

However, in spite of the above-mentioned limitations, ligation of a major coronary artery still remains the oldest and most widely used method of producing experimental acute myocardial ischemia in a variety of laboratory animals. A main criticism of this method also includes the high mortality rate and the difficulty in obtaining standardized lesions. Many of these shortcomings have been partially obviated by improvements in techniques so that in certain works the resulting lesions and the sequence of myocardial changes are considered similar to those found in man after an acute cardiac event recorded as myocardial infarction.

F. Regression of Experimentally Induced Lesions

1. General Observations

Perusal of the literature demonstrates that coronary atherosclerosis is a basically progressive disease, regression occurring only occasionally, as was documented by coronary angiography about 15 years ago.[375,376] Cases in which the arteriograms showed regression of obstructions represented approximately 4% of the nonsurgical patients in which sequential coronary arteriographic studies were performed. This led to the conclusion that the regression of coronary atherosclerosis is possible, but spontaneous regression is rare and seems to be mainly based on partial thrombolysis. This apparent regression could not be clearly related to clinical parameters.[377]

In spite of these uncertainties, a general consensus exists that in the treatment of coronary atherosclerosis, the main task is not only to prevent progression, but also to stimulate regression of lesions.

Although regression of coronary atherosclerosis has been repeatedly documented by sequential contrast arteriography in humans, the correspondence of regression in animals submitted to cholesterol-rich diets with the regression recorded in humans needs to be established, since it depends heavily on the etiologic correspondence of experimental and human coronary atherosclerosis.

Regression of the size of experimentally induced lesions, whether defined in terms of increasing arterial lumen, decreasing lesion cross-sectional area, or decreasing lesion cholesterol content, has been shown to be attainable in many animal models.

The investigators begin by producing lesions in a large group of animals by feeding them an atherogenic diet and elevating blood cholesterol levels. After a period of time deliberately proposed by each team of investigators, a portion of the animals are killed and the baseline lesions recorded. These lesions were to be regressed, since it was tacitly accepted that lesions

identical to those of the killed animals were also present in the nonkilled. These nonkilled animals were then treated with low cholesterol diets with or without drugs and killed after various additional periods. The lesions submitted to regression were then compared to lesions recorded in the animals fed atherogenic diets and the results of this comparison were used to demonstrate regression.

In some experimental models blood cholesterol levels do not immediately return to normal when the atherogenic diet is changed to a treatment regimen and lesions may continue to progress rather than regress. In other experimental models, success of the treatment could be easily overestimated if the increase in luminal diameter due to age-related augmentation in vessel size, or due to segmental dilation that occurs in atherosclerotic vessels were taken as plaque regression.

The regression of atherosclerotic lesions produced by a cholesterol-rich diet involves at least three possible mechanisms:[378] (1) breakdown of lipid by enzymatic activity, (2) removal of lipid by phagocytosis and cell transport, and (3) the action of plasma lipoproteins as transport vehicles, LDL and VLDL being mainly responsible for transporting cholesterol into coronary arterial wall, while HDL is concerned with the removal of cholesterol from the tissues.

The most dramatic and consistent depletion in experimentally induced atherosclerotic lesions is that of the lipid content. The disappearance of intracellular lipid is often associated with that of lipid-filled cells or foam cells. As concerns extracellular lipids, they can be mobilized only very slowly, particularly if they are associated with necrotic material or appear as cholesterol crystals. Nevertheless, it was possible to demonstrate not only the disappearance of lipids, but also a reduction of the degree of luminal narrowing in the coronary arteries.

Substantial regression even of advanced lesions was related to reduced level of serum cholesterol to a minimum that prevails in animals or humans consuming a lowfat, low-cholesterol diet. In human subjects and rhesus monkeys, this appears to be about 150 mg/100 mℓ, a level at which much of the lipid disappears from the plaques.[379] This view is also supported by indirect retrospective and poorly controlled data, such as the study carried out on individuals dying of wasting diseases, with a terminal weight loss of up to 45 kg. These were compared with corresponding coronary arteries from autopsied individuals who had little or no terminal weight loss. The regressed plaques of the wasting disease group (mainly cancer patients) looked similar to regressed plaques in experimental animals.[380]

An additional, indirect proof of lesion regression in coronary atherosclerosis is based on the disappearance of large cutaneous or tendon xanthomas of patients with familial hypercholesterolemia during cholesterol-lowering treatment.

A decrease in the LDL content in coronary artery lesions may appear even without significant reduction in lesion size and may be responsible for the diminution in the number of foam cells.[381]

The composition of the glycosaminoglycans in the arterial wall also seems to play a role in the induction and regression of experimental atherosclerosis; plaques which show evidence of regression have increased concentrations of hyaluronic acid and heparan sulfate, while dermatan sulfate and chondroitin-6 sulfate decreased.[382]

When we look at a lesion that is thin and lipid-poor, we cannot be sure that it was previously thicker and richer in lipid since we are able to visualize the same lesion only once during its natural history. Likewise, we cannot be sure that we may have selected by accident for histological study thicker and lipid-richer lesions from the induction group, or thinner and lipid-poorer lesions from the regression group. This may lead to aspects of pseudoregression.[383] Moreover, it is possible that by accident, on investigating animals more resistant to a cholesterol-rich diet in the regression group, we may find the same pseudo-regression. We can prevent so many possible errors by using large numbers of animals per

group, studying multiple histological samples removed from standardized topographic sites, and by using quantitative methods. In addition, our study must be centered on lesions consisting primarily of foam cells, monocytes, and little collagen, since such lesions are capable of significant loss of histological and chemical intracellular lipid when the blood cholesterol level is very low.[383]

When regression appears, its speed is slow or very slow, between 1 and 4 years, in contrast to the relatively rapid adsorption of lipid from subcutaneous sites which requires only 6 to 12 weeks.[384] The relative slowness of regression of atherosclerotic lesions when compared with subcutaneous lipid resorption may be related to a limited capacity of arterial intima to metabolize cholesterol, particularly in the presence of a perturbed balance between LDL and HDL. This is associated with a prevalence of cholesterol crystals and with the relative absence of macrophages and reticulo-endothelial phagocytes. The relative slowness of regression of atherosclerotic lesions might also explain why in many models, loss of lipid did not vary directly with the blood cholesterol level, in spite of the fact that cholesterol was the principal lipid lost in regression. There is no capillarization of the experimentally induced plaques, except very late, when the media is largely destroyed. Thus, unlike a granuloma anywhere else in the body, the content of an atherosclerotic plaque cannot be completely eliminated and especially rapidly eliminated. Both large necrotic centers including disintegrated cells and various debris and the thick fibrous cap surrounding the necrotic mass, represent tissues with little prospect to regression. Conclusions from the most critical experimental studies indicate that incipient or young lesions are capable of significant regression, but there is no clear evidence that advanced or complicated plaques will regress in any species.[383] An intriguing problem is to separate regression from remodeling and healing, since all may coexist in the same vascular segment: when regression prevails, this is reflected by the diminution in volume or mass of the plaque that would result in augmentation of the luminal diameter and increased arterial flow rate.[385] Arterial smooth muscle cells are able to produce collagen fibers and even macromolecules involved in the onset of the thick, hyalinized fibrous cap of advanced atherosclerotic plaques, but are also able to degrade and disintegrate collagen fibers.[386] This was regarded as one of the mechanisms that accounted for the disappearance of collagen fibers from regressing lesions.

In summary, in the past 2 decades considerable progress has been made toward understanding of the events that take place in regression of established atherosclerotic lesions in a variety of experimental models. Based on these findings, an evaluation was also made on the potential for regression in man: what kind of atherosclerotic lesions could be expected to regress in human beings and the conditions under which regression would be most likely to occur. Questions have been raised as to the validity of findings furnished by experimental models, due to the fact that atherosclerosis in animals is usually produced by exaggerating a single factor — hypercholesterolemia. The relevance of regression data on animals to regression in humans depends heavily on the etiologic correspondence of experimental and human atherosclerosis. As long as this etiologic correspondence could not be demonstrated "the results appear as diverse as the authors".[387]

2. Selected Examples

a. Rabbit Model

The first studies on atherosclerosis regression were done with rabbits, a gradual disappearance of lipids accumulated in lesions being detected. These lesions were converted from large lipid-rich plaques into small fibrous plaques during a period of 2 to 3 years.[388] On light microscopic examination, regression was characterized by a progressive disappearance of lipid-filled cells and their replacement by collagen and elastic fibers. Other authors reported conflicting results, such as lack of reversibility of rabbit experimental atherosclerosis after discontinuity of cholesterol feeding, or even the finding of more advanced atherosclerotic

lesions following prolonged periods of cholesterol-free diets.[389,390] This led to the conclusion that of all species tested, the rabbit shows minimal potential for regression.[390] This may be due to the fact that withdrawal of the high cholesterol diet does not lead to an immediate decrease of the blood cholesterol level in rabbits, since in these animals experimental atherosclerosis develops as a storage disease, similar to true lipoidosis. Consequently, the excess of cholesterol accumulated in various organs and tissues continues to filter into the blood flow, thus protracting hypercholesterolemia for months, and during this period arterial infiltration with blood lipid still occurs.

In some experiments, rabbits fed an excess cholesterol diet for 2 to 3 months and then allowed to survive for 2 years on a cholesterol-free diet showed more extensive and severe lesions than rabbits sacrificed at the end of the period of cholesterol feeding. The difference consisted particularly in more fibrous transformation of the lesions and a basal displacement of the lipid in the plaque which may be due to the additional hypercholesterolemia after the return to a cholesterol-free diet.

Obvious evidence of regression in rabbit atherosclerosis has been reported only for lesions induced by shorter exposure (2 to 3 weeks) to a high-cholesterol diet, until the blood cholesterol level reached 1000 mg/100 mℓ. The discontinuity of cholesterol feeding in animals returned to normal diet and killed at intervals up to 300 days resulted in various degrees of lesion reversibility.[391]

In spite of many uncertainities, the rabbit model was largely used to test the direct action at the arterial wall level exerted by hypolipidemic drugs associated with cholesterol-free diets. These drugs act in a very complex manner by lowering blood cholesterol level, inhibiting cholesterol synthesis, increasing cholesterol metabolism, inhibiting cholesterol reabsorption, interfering with lipoprotein metabolism, etc. The direct action at the arterial wall level exerted by the hypolipidemic agents upon either synthesis or hydrolysis of cholesteryl esters has been repeatedly emphasized.[392] Changes of the cholesteryl ester synthetase to cholesteryl ester hydrolase ratio to lower values by drug treatment should be regarded as an important indirect evidence of regression; this indicates that processes of degradation prevail over those of synthesis. Hyperoxia may produce the reversal of atherosclerotic lesions in rabbits, as it diminishes the content of total cholesterol, triglycerides, and phospholipids.[393] More complex therapeutic combinations, such as lowfat diet-hyperoxia and cholestyramine or lowfat diet-hyperoxia and estrogen, have proven to exercise a positive synergistic effect in experimental atherosclerosis regression.[394] There are grounds for thinking that the effect of some of these drugs is partly related to their ability to stimulate phagocytic cells in lipid transport.[395,396]

b. Avian Models

Chicken and pigeons were largely used for regression studies. In chickens, during regression, blood cholesterol levels decreased from 1000 mg/100 mℓ to only 150 mg/100 mℓ as a result of cessation of cholesterol feeding. This rapid and constant return of blood cholesterol to normal values was related to more evident results on regression obtained in chickens than in rabbits. Indeed, regression of coronary atherosclerosis in chickens can be documented after only 2 to 3 weeks of cessation of cholesterol feeding, the extent of regression being directly related to the total blood cholesterol concentration during the regression period.

Pigeons have also been used extensively for the study of regression of cholesterol-induced atherosclerosis; a naturally occurring regression is also observed secondary to ileal bypass. In both experimental and spontaneous atherosclerosis regression, an increase in the ratio of cholesteryl ester hydrolysis to cholesterol ester synthesis was recorded, associated with a reduced rate of cholesterol esterification.[397] After the lesions have lost a substantial portion of their lipid content, an augmentation in the number of collagen fibers was observed, suggesting that the loss of foam cells stimulates a fibrotic reaction. This could lead to almost

complete replacement with collagen fibers and proliferating smooth muscle cells of the space previously occupied by foam cells. This could also lead to changes difficult to record as concerns the variations in the luminal diameter, the extent to which the coronary lumen is able to return to normal values being limited by the neoformation or collagen fibers, and proliferation of intimal smooth muscle cells.

c. Swine Model

This model can be used to demonstrate not only a loss of foam cells, but extracellular necrotic material after regression diets.

During the 4-month induction period, typical complicated plaques develop, with extensive lipid deposits, necrosis, hemorrhage, mural thrombi, and calcification. On the other hand, in the regression group of animals fed mash the following 14 months, only flat lesions are present, lacking necrosis, hemorrhage, or thrombosis. Biochemically, less phospholipid, total, and especially esterified cholesterol were found in the lesions of the regression group and both morphological and biochemical data could be interpreted as evidence that complicated atherosclerotic plaques may regress to a certain extent.[398] The results were improved if hypolipidemic drugs such as clofibrate was added to the cholesterol-free diet.

d. Nonhuman Primate Model

The interest in regression studies is considerable since in these species the cholesterol metabolism, the blood lipoprotein pattern, and the histogenesis of certain atherosclerotic lesions bear close resemblance to those in humans.

The information on regression was mainly obtained from both rhesus and cynomolgus monkeys. During the regression period blood cholesterol levels decreased from more than 700 mg/100 mℓ to 140 mg/100 mℓ and the average luminal narrowing from 58 to 18%. Microscopically, the lesions lost their fatty components and showed a condensation of collagen fibers. Biochemical analyses revealed a loss of free and esterified cholesterol. A greater loss of esterified than of free cholesterol is usually recorded.[399,400] In addition to cholesterol, cellular and necrotic material was lost and the lesions were reduced in size. However, many of the advanced lesions still retained excess cholesterol, many collagen fibers, and areas of calcium deposition; this could indicate that there was no tendency to regression.

In monkeys with a blood cholesterol level of 200 to 300 mg/100 mℓ, similar to that of human beings considered at risk of coronary heart disease, the aspects of regression were variable; at 300 mg/100 mℓ there was little or no decrease in lumen narrowing, while at 200 mg/100 mℓ there was a considerable decrease.[397] Some lesion areas previously occupied by lipid may be replaced by fibrotic zones and such replacement may severely limit regression of the lesion. Moreover, at 300 mg/100 mℓ, a further increase in the lesional cholesterol content suggested continued progression.

The regression potential seems to depend on both initial lesion severity and on blood cholesterol levels. In certain studies there was no reduction in lumen area — the lesions became less fatty, due principally to a reduction of intracellular lipid. On the other hand, there was little loss of extracellular lipid deposits, cholesterol clefts, and necrotic debris, all associated with an increase in collagen fibers. This may reflect an augmented synthesis of collagen macromolecules as part of a generalized repair process and/or condensation of preexisting fibrils and fibers. Chemical studies detected increase, no change, or decrease in the content of collagen during regression. Increase in collagen content was mainly recorded in complicated plaques with a thick fibrous cap, and the accumulation of newly formed collagen fibers was sometimes so extensive that it could largely replace the space previously occupied by foam cells. Therefore, no visible diminution in lesion size could be observed, but only a tendency to produce diffuse and very thick intimas. On a group basis, however,

the differences between atherogenic and nonatherogenic diets appeared highly significant. In the monkeys fed an atherogenic diet the lesions covered almost the whole coronary intimal surface, exhibiting large lipid deposits, necrotic masses, fibrous cap, altered internal elastic membrane, and calcifications. In the monkeys fed an antiatherogenic diet, the lipid deposits were very small and located mainly on the internal elastic membrane. During the regression period, blood cholesterol levels and the LDL/HDL ratio returned to normal values, the number of lesions was reduced to two thirds of those existing at the end of the high-fat, high-cholesterol diet period. When blood cholesterol level was maintained at approximately 175 mg/100 mℓ for the rather long period of 18 months, changes considered as regression were recorded even in advanced obstructive atherosclerotic lesions.[396]

As in other experimental models, lowering of the blood LDL level following termination of a hypercholesterolemic diet could be followed by a reduction of LDL content in coronary artery lesions, even without significant diminution in lesion size. This reduction of lesion LDL content was associated with a diminution in the number of foam cells.[401] This suggests that removal of hypercholesterolemic stimulus can affect beneficially preestablished atherosclerotic lesions, by directly or indirectly reducing the number of foam cells in such lesions.

In addition, following drastic reduction in marked hypercholesterolemia induced in monkeys by diet, the following changes were recorded: restored integrity of the endothelium lining the plaques, absence of smooth muscle cell proliferation, decrease in the number of smooth muscle cells, and decrease in the amount of extracellular lipid and necrotic debris. On the other hand, residual fibrosis may become prevalent and produce shrinkage of the lesion and an increase in the lumen diameter. A biochemical ''scar'' may also be recorded, consisting of a residual content of cholesteryl ester greater than of free cholesterol at the end of the regression period. This residual cholesterol was not susceptible to further depletion by dietary means and persisted as such even after very long regression periods.[400]

Premature squirrel monkeys treated with chondroitin-4 sulfate (6 days/week, 20 mg per animal) exhibited an important regression of atherosclerotic lesions which had been previously induced by cholesterol-rich diets. Following the regression period, analysis of the arterial lipid content revealed the same lipid pattern as in control animals submitted to a standard diet.[402] Studies on arterial wall glycosaminoglycans during experimental regression of atherosclerotic lesions in cynomolgus monkeys revealed elevation of heparan sulfate and hyaluronic acid, whereas chondroitin sulfates followed an opposite trend.[403]

The results obtained in monkeys during regression studies cannot be extrapolated from one species to another since they may not appear valid.[271] Cynomolgus seems to be a species adequate to experimental induction and regression of atherosclerosis, showing more striking arterial wall changes.[404] However, the high level of blood cholesterol attained in monkeys during relatively short induction periods somewhat limits the applicability of these findings to man, and reduces the whole problem of atherosclerosis etiopathogenesis to hypercholesterolemia. On the other hand, it is not possible to overlook the fact that in nonhuman primates proper manipulation of the diet could cause obstructive atherosclerotic lesions to shrink and nonobstructive atherosclerotic lesions to disappear.[224] Advanced atherosclerotic lesions of nonhuman primates respond more favorably when blood cholesterol levels were reduced to a minimum that prevails in people consuming a lowfat, low-cholesterol diet, suggested to be about 150 mg/100 mℓ.[385]

The question of whether treatment of arterial hypertension would slow down the rate of atherosclerosis progression or would promote regression is also an attractive subject of research using nonhuman primate models.[405] Blood pressure levels were increased in cynomolgus monkeys to an average value of 180/120 mmHg by coarctation of the aorta and the blood cholesterol level was augmented to about 510 mg/100 mℓ by feeding an atherogenic diet. This diet was then withdrawn and some subgroups received, in addition, antihypertensive therapy which reduced blood pressure to an average value of 130/80 mmHg. Com-

bined treatment of hyperlipidemia and hypertension was more effective on lesion regression than treatment of hyperlipidemia alone.[405]

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Chapter 3

FACTORS AND MECHANISMS INVOLVED IN CORONARY ATHEROGENESIS

I. SUGGESTED ATHEROGENIC ROLE OF HEMODYNAMIC STRESSES

A. Introductory Remarks

An objective analysis of the factors and mechanisms involved in coronary atherogenesis shows that atherosclerotic lesions may develop in subjects with very high, but also with very low, serum cholesterol levels, hypertensive and normotensive individuals, heavy smokers and nonsmokers, diabetics and nondiabetics, cases submitted and not submitted to important psychosocial stresses, subjects with or without genetic abnormalities of lipid, protein, or carbohydrate metabolisms, etc. There are, of course, significant differences concerning the period of onset, extent, and severity of atherosclerotic plaques due to the action of particular diseases or risk factors, compared to "control" subjects. This means that the above-mentioned factors and mechanisms *aggravate the natural history of coronary atherosclerosis,* but are not *sine qua non* atherogenic agents.

In the human coronary arterial tree the sine qua non *factor for atherosclerotic involvement seems to be a special pattern of hemodynamic stress,* present in vessels larger than 1 mm in which a mean pulsatile pressure higher than 100 mmHg exists. In such conditions, *the onset of atherosclerotic plaques does not require the particular microarchitecture of an arterial wall, they can also develop in veins grafted into the arterial system and in the neointima of arterial prostheses made of plastic material.* This demonstrates that the *sine qua non* factor for the onset of atherosclerotic plaques is only a particular hemodynamic pattern and all pathogenetic mechanisms involved in coronary atherogenesis are mediated, at least in part, by mechanical forces associated with blood pressure and flow.

Hemodynamics, which have been previously regarded as being a minor localizing factor is considered by certain scientists to play a major role as an etiologic agent of human coronary atherosclerosis.[1] This assumption is supported by the observations that:

1. The greater the caliber of the vessel the more severe the atherosclerotic involvement
2. The greater the arterial pressure the more severe the atherosclerotic involvement
3. The greater the number of branching points and other changes in the vessel geometry the more severe the atherosclerotic involvement

The following conclusion is worth mentioning: "The evidence accumulated suggests that it is vascular geometry, and the way it influences the local detailed flow properties, which is the primary determinant of a hemodynamic effect on the arterial wall and in the initiation of atherosclerosis."[2]

The microarchitecture and macromolecular composition of the coronary arterial bed is established by genetic factors in the presence of marked, moderate, or mild hemodynamic stresses, being particularly influenced by mechanical tension, and especially the rates of tension change. The coronary arterial wall must be regarded as an active and responsive muscular and connective tissue, permitting adaptation of its structure and composition to long-term mechanical demands, the role of arterial fluid dynamics being quite possibly extremely subtle.[3]

Because of the limitations of equipment, instrumentation, and access, only few detailed characteristics of hemodynamic stress that are important in coronary atherogenesis are available for measuring in normal and pathologic conditions. This may be also related to the

very limited number of investigators dealing with such studies, as compared to the myriad of studies dealing with changes in lipid metabolism. Moreover, all scientists working in the field of tissue culture and with various other in vitro models overlook the influence of hemodynamic stress. Also of note is the observation that the particular hemodynamic pattern which exists in human coronary arteries is difficult to analyze in small laboratory animals with an intramyocardial course of the major coronary arteries or with an anatomical branching pattern different from that of human beings.

The human coronary arterial wall normally operates under a peculiar oscillating state of stress applied to the vessel wall by intravascular pressure and by longitudinal traction. The major coronary arteries (except the left main coronary artery) have many branching points able to transform laminar into turbulent flow; in addition, they are rhythmically subject to torsion, spiral twisting, and bending with every myocardial contraction and are rhythmically compressed by the contracting heart muscle. In contrast to other arterial beds which show a more or less constant resistance to flow during a cardiac cycle, the coronary arterial tree has a fluctuating resistance, dominated by the left ventricular contraction which acts by impeding flow through the left ventricular wall during systole. Likewise, in contrast to other arterial beds in which a normal or undisturbed flow pattern can be revealed, in the coronary arteries a disturbed pattern is often present, characterized by an elevated shear stress, turbulence, vibrational phenomena, boundary layer separation, and alteration in mass transport across the coronary wall, etc.

If we accept the view that hemodynamic variables influence the onset, rate and topography of atherosclerotic lesions, as well as their progression toward lesions of possible clinical significance, then *the diversity in the branching patterns of human coronary arteriees and implicity of the flow patterns may significantly contribute to the variance in atherosclerotic involvement and myocardial clinical manifestations.*

The supposition that hemodynamic forces are important in the initiation and localization of atherosclerotic plaques originated from repeated observations that such lesions do not occur randomly in the coronary arterial tree. Certain sites and geometric configurations have a significantly higher susceptibility of developing plaques than do other sites.

In regions where smooth muscle cells proliferate to give rise to fibromuscular plaques, it might be assumed that the coronary arterial wall was stressed beyond the limits of its optimal adaptability; intimal thickening is also predictable according to the laws of hydrodynamics, and the severity of atherosclerotic involvement may be strongly related to the extension and degree of intimal thickness. Studies carried out in our laboratory revealed a strong influence of the anatomical branching pattern of the coronary arteries on the onset, microarchitecture, and severity of the thickened intima.[4-6] These are only indirect arguments but they demonstrate that special hemodynamic features seem to exist in the human coronary arterial tree in the ability to produce very early during the ontogenetic evolution both thickened intimas and fibromuscular plaques. In other arterial beds with vessels of similar size, such intimal development is absent or very late and is, by comparison, trivial.

Therefore, the results of our studies, like those of investigations carried out by Friedman and his team,[7,8] strongly suggest that *vascular geometric features represent a potential atherogenic factor and in addition a risk factor for coronary heart disease.* This concept needs to be further developed.

The coronary arterial tree is well suited to demonstrate that at each site mechanical stresses are inducers of the local changes that adapt vascular microarchitecture, thickness, and caliber to the forces in operation. The smooth muscle cells of the coronary arteries seem to be sensitive to these stresses and capable of responding to the hemodynamic and mechanical forces with counteracting mechanisms. These cells produce connective tissue components and myofibrils in a manner which allows the removal of stressing forces from the cells, negative feedback mechanisms being involved in the complex regulations.[9] The coronary

smooth muscle cell is considered able, in the presence of a sudden application (acceleration) of tension, to trigger an equally sudden active shortening or counteracceleration. When the tension prevails, smooth muscle cells are stimulated to synthesize mainly collagen; when the rate of change in tension prevails, elastin macromolecules are mainly produced; when acceleration of tension prevails, an increase in the number of intracellular myofibrils could be recorded. Briefly, the smooth muscle cells appear to use such negative feedback mechanisms to adjust the strength of each segment of the coronary arterial tree to local changes, induced by hemodynamic and mechanical stresses. In the light of this view, the occurrence of an elastic hyperplastic sublayer in a thickened intima would reflect an important rate of change in tension in the respective region induced by pulsations; the newly formed elastic fibers are able to relieve the cell of the oscillating forces. Likewise, the number of collagen fibers in a given arterial segment will increase if the tension augments for long periods of time; in addition, smooth muscle cells are able not only to produce collagen fibers, but also to reduce the preexisting number of these fibers at those sites where collagen fiber accumulation is excessive relative to the magnitude of the stretching forces.[9]

The same lipoprotein level and pattern exist in the flowing blood which passes through various segments of the coronary arterial tree, and yet the onset of atherosclerotic lesions and their progression toward advanced forms differs regionally. This would indicate that local hemodynamic factors will determine if an atherosclerotic plaque will appear and when and where it will develop. There still is an open question as to the pathogenetic mechanisms through which hemodynamics become atherogenic. A number of flow-endothelium interactions have been suggested to play a main role as indicated in other sections in this chapter. Their analysis is a difficult task, since the use of model systems in which tubes are of constant diameter and the flow is constant are of limited value for clarifying the conditions present in the human coronary arterial tree. Models can only give a vague impression of what is really happening along a branching artery with pulsatile flow, changes in elasticity and muscular tone with intercalated vascular segments, sphincteric microarchitecture, and many other peculiar morphologic features which exist in human coronary arteries. Our knowledge is too limited and the interactions are too complicated to be described in a general way, based on data offered by in vitro models. The methodological limitations of current techniques, the existence of a resting coronary flow, the variability of regional distribution of coronary branches, and even the fact that blood flow is complex and difficult to analyze accurately, might explain why we have hesitated to present certain of the mathematical models proposed. On the other hand, comparative studies are too closely related to species differences.[10]

Blood in the coronary arterial tree does not behave as a Newtonian fluid; it is pulsatile and not steady and the coronary arteries have a variable anatomical branching pattern, a variable degree of extensibility from one vascular segment to the other, and exhibit many branch sites, curves, and taperings. It is tempting to presume that where an artery is "tethered" by branches or by firm attachments to underlying myocardium, the shearing stress might be accentuated; an augmented susceptibility to atherosclerotic involvement was also recorded when the pulse and systolic pressure were high, as in subjects with arterial hypertension or submitted to important and repeated catecholamine release. Emphasis is also placed in certain studies on the atherogenic role of turbulent flow. Turbulence is likely to arise just within the branching areas in points where atherosclerotic plaques usually develop.[11] Hemodynamic stress also influences the coronary artery wall as a metabolizing tissue with particular features in the supply of nutrients and oxygen, in the removal of metabolites and in changes in intercellular space resulting from accumulation of abnormal quantities of plasma components. Under normal conditions, mechanical forces related to pressure tend to deform the coronary artery wall longitudinally (in the direction of blood flow), radially (outward or perpendicular to the vessel wall), and circumferentially (parallel

or tangential to the wall). There are tensile stresses acting in both circumferential and longitudinal direction, with a radial pressure graded from within-outward, and shearing stresses at the vascular interface, related to the friction of the adjacent blood flow.

The physiological response of the coronary arterial wall to hemodynamic stresses may be related to functional changes and ultrastructural changes, the most important for atherogenesis being those associated with an augmented endothelial permeability. Atherogenesis seems to be accompanied by the incapacity of the arterial wall to adapt to hemodynamic stress in such a way as to maintain effective transintimal passage and clearance of plasma components. The manner in which hemodynamic stress may transform an orderly intimal adaptive response into a self-perpetuating accumulation of lipid and fibrin, associated with cell necrosis, sclerosis, and hyalinization, remains a subject for future investigations. There may be mass transport hemodynamic parameters affecting the coronary arterial wall which have not as yet been clearly delineated. Some studies suggest that under the influence of certain hemodynamic stresses weak Van der Waals bonds may be broken down in intimal fibers and ground substance and this may result in lowering the potential energy barrier for diffusion molecules.[12] Other studies are centered on the "fatigue" produced by local hemodynamic stress.[13] As applied to engineering materials, fatigue is a state in which fractures occur suddenly due to a reduced tensile strength, associated with other types of molecular alterations developing after exposure to repeated alternating stress; in biologic material this would lead to various types of reparative processes. Such reparative processes often occur in the coronary arteries, distensible vessels which are deformed viscoelastically under the pressure variations associated with pulsatilability of the flow and heart contractions. Fatigue is a state that may more frequently appear in the coronary than in other arterial beds because the coronary arterial tree has an asymmetrical geometry which could produce asymmetry in the velocity patterns; the geometrical tapering of the major coronary arteries and the many branch sites affect the amplitude, as well as the speed of propagation of the arterial pulse wave, giving rise to wave reflection which can propagate in both proximal and distal directions.

An important effect related to arterial wall fatigue may be the constraint imposed upon the motion of an arterial segment of the major coronary arteries by the surrounding tissue, since this could significantly influence the amplitude and speed of propagation of the coronary artery pulse wave. The amplitude and speed of propagation of the coronary pulse wave also change dramatically when the flow passes from a major coronary artery into an intramural branch. The absence of intimal thickening and plaque development in intramural coronary arteries and in areas covered by myocardial bridges along the course of the left anterior descending artery advocate for the importance of flow changes in atherogenesis and resistance to atherosclerotic involvement.

A largely accepted view is that an intensified hemodynamic stress might become an atherogenic agent, this view being supported by the results of many experimental models. An intriguing question remains if a hemodynamic stress considered between physiological limits could also be atherogenic. Moreover, if the flow persists in certain coronary arterial segments at a reduced rate, the intensity of hemodynamic stresses is also drastically reduced, but this is not associated with an augmented resistance to atherosclerotic involvement. Very often this reduced intensity and duration of hemodynamic stresses could lead to an intimal proliferation, particularly in the subendothelial region. This proliferation considerably narrows the artery lumen. An eloquent example is the postnatal progressive closure of ductus arteriosus. In some experimental models, ligation of an artery followed by a hemodynamic stress of a very reduced intensity led to intimal proliferation and vascular lumen narrowing. One can speculate that in the coronary arterial bed there are also local peculiar hemodynamic conditions associated with reduced blood flow, which stimulate subendothelial smooth muscle cell proliferation, intimal thickening, and luminal narrowing. The primary disturbance

may be prolonged segmental constriction leading to reduced blood flow or the presence of a stenotic plaque which produces an important diminution of blood flow.

In essence, although the role of hemodynamics in atherogenesis is widely accepted, the most important hemodynamic factor(s) related to the onset and progression of atherosclerotic plaques have not yet been identified with certainty and almost every hemodynamic phenomenon is proposed at one time or another. The view that we must begin to study more carefully new parameters of the mechanisms whereby hemodynamics affect the arterial wall[14] is also worth considering. To elucidate the relation between coronary blood flow and coronary atherogenesis, a considerable amount of work, both theoretical and experimental, is still necessary.

B. Selected Examples

The pulmonary artery, normally exposed to a low blood pressure, does not develop atherosclerotic plaques; even if serum cholesterol levels are very high (400 to 500 mg/100 mℓ) and for very long periods of time, the wall of the pulmonary artery appears resistant to the onset of such lesions. On the other hand, the same vessel may develop severe atherosclerotic plaques, even in subjects with less than 200 mg/100 mℓ serum cholesterol levels in the presence of pulmonary hypertension. Many investigators have emphasized the view that an increased intraluminal pressure is the unique factor which can be associated with atherosclerotic involvement of the pulmonary artery.[15,16] The first analytical investigation in this field, made more than 70 years ago,[17] revealed that the elastic tissue of the aorta appeared altered before or immediately after birth, whereas the elastic laminae of the pulmonary artery did not shown similar changes. Intimal thickening was constantly detected in the aorta immediately after birth, but it was only the pulmonary artery of elderly individuals that presented similar thickening. During systole, the intraventricular pressure changes from near 0 to 120 mmHg in the left ventricle and then in the aorta where the elasticity of the vessel and the peripheral resistance to flow is associated with a diastolic pressure of approximately 80 mmHg. In the right ventricle, the intraventricular pressure during systole changes from 0 to only 25 mmHg and the pulmonary resistance is about 10 mmHg. To sum up, the pulse pressure in the aorta and major coronary arteries is 120/80 mmHg, and this pressure is considered able to act as an atherogenic agent; in the pulmonary artery the pressure is 25/10 mmHg, having no atherogenic character.

In subjects with the left main coronary artery originating from the aorta and the right coronary artery originating from the pulmonary artery, **the phasic pressure cycle** is approximately 120/80 mmHg in the left main coronary artery, left anterior descending, and left circumflex arteries and only 25/10 mmHg in the right coronary artery. Post-mortem examination of such cases revealed severe atherosclerotic involvement of the left main coronary artery, as well as of the left anterior descending and circumflex arteries and an unexpected resistance to atherosclerosis of the right coronary artery. If the right coronary artery originated from the aorta and the left main coronary artery from the pulmonary artery, the right vessels appeared intensely susceptible and the left ones nonsusceptible to atherosclerotic involvement. Such observations which demonstrate the existence of a clear correlation between a certain level of intravascular pressure and the onset, extent, and severity of atherosclerotic involvement in the coronary arterial tree were reported more than 2 decades ago.[18]

Calcific aortic valvular stenosis usually produces diminution of the diameter of the coronary ostium. When this diminution is marked, it is associated with a reduction of the blood flow, pulse pressure and other hemodynamic stresses, and a very slow progression of atherosclerotic plaques compared to subjects of similar age and sex, without calcific aortic valvular stenosis.[19]

An accelerated coronary atherosclerosis with rapid onset and progression of plaques

was recoreded in about $^1/_3$ of children with nephrotic syndrome, hypercholesterolemia, and hypertension; the remaining $^2/_3$ were normotensive and did not show the rapid onset and progression of atherosclerotic plaques, in spite of similar high serum cholesterol levels.[20]

Patients presented with **coarctation of the aorta,** leading to severe hypertension in the brachial, but not in the other arterial beds. Post-mortem examination revealed extensive atherosclerosis in the areas submitted to hypertension, a clear delineation being possible between hypertensive and nonhypertensive arterial beds. Experimental coarctation of the aorta may produce in thyroidectomized dogs fed cholesterol-rich diets a vascular area of susceptibility to atherosclerosis which corresponds to the area of hypertension including the coronary arterial tree. The atherosclerotic lesions develop only when the degree of constriction is such as to produce a pulse pressure above the coarction that is 30 mmHg or higher. These are considered crucial cases and experiments because they demonstrate in the same subject or laboratory animal *the onset of atherosclerotic plaques only in areas with elevated blood pressure.*[21,22]

Atherosclerotic plaques were revealed in **arteriovenous fistulae** or in limited areas of the venous system showing an abnormally increased intraluminal pressure.[23,24]

Vein autografts placed into arteries developed a thickened intima in which atherosclerotic plaques progressively appeared.[25] Autografts of jugular vein placed in the abdominal aorta of hypercholesterolemic animals showed intimal thickening and onset of atherosclerotic lesions.[26] Even Dacron prostheses inserted in the aorta and exposed to hypercholesterolemia developed a pseudo-intima in which there occurred changes considered to be atherosclerotic lesions.[27] Finally, the rapid and extensive development of atherosclerotic plaques in saphenous vein grafts implanted into the coronary arterial tree was presented in many reports and is more largely discussed in the next pages.

Arteriovenous shunts established in patients undergoing chronic hemodialysis for kidney failure frequently occlude because of obstructive atherosclerotic plaques developing from successive intramural thrombi in the vein, immediately distal to the shunt. Venous segments removed at the time of shunt revision showed marked intimal thickening with fresh luminal thrombus, collection of lipid-filled cells, and even cholesterol clefts. Changes similar to those detected in the veins of arteriovenous fistulas in hemodialysis patients were produced in the vein walls of experimentally constructed carotid-jugular arteriovenous aneurysms.[28] Many other results advocate for the assumption that hemodynamics is not merely a localizing factor in atherogenesis, but is capable of producing lesions in a vessel subjected to hemodynamic stress of arterial type. These atherosclerotic lesions develop without injurious devices, abnormal diets, and pharmacologic agents.[29,30] The distribution of atherosclerotic lesions around the bifurcation **of the left main coronary artery** was shown to be related to the geometry of the respective bifurcation. Analysis of the silicone rubber casts showed that atherosclerotic plaques have a distinct pattern with a high frequency on the outer walls of the bifurcation and at the inner curvature downstream from the bifurcation.[31]

More than 3 decades ago it was observed that **myocardial bridging** of the proximal segment of the left anterior descending artery is associated with an increased susceptibility to atherosclerotic involvement and luminal narrowing in the premural portion and a reduced susceptibility to atherosclerotic involvement in the intramural portion of the same vessel.[32] Myocardial bridges which significantly limit the distension of the left anterior descending artery produced by the pulse wave protect the intramural portion of the vessel not only from obstructive lesions, but also from early atherosclerotic involvement. These obstructive lesions develop just before penetration of the artery into the muscular tunnel, whereas in the intramyocardial course the vessel shows a thin wall without important pathologic changes. The absence of severe intimal thickening and plaque development in segments of the coronary arterial tree which show an intramural course clearly indicates that limitation of distension, or the increased tissue support by the encassing myocardium or both, transform a susceptible arterial wall into a wall resistant to atherosclerotic involvement.

Anatomic variations of the coronary arteries around the crux of the heart are sometimes associated with variations in the susceptibility to the onset of atherosclerotic plaques and their progression lesions of possible clinical significance. In certain works, a higher incidence of ventricular conduction disturbances and a greater incidence and severity of obstructive atherosclerotic lesions is mentioned in subjects with the predominant left, as compared to subjects with the predominant right, coronary circulation.[33] These results suggest an anatomically disadvantaged status of the predominant left coronary circulation as regards ventricular conduction abnormalities and coronary atherosclerosis.

A geometric or anatomic risk factor acting by means of particular hemodynamic stresses was also indirectly demonstrated in our laboratory since we observed a close association between certain anatomical branching patterns and atherogenesis.[4] We were able to detect in one out of every three to four unselected subjects minor deviations from the common type of distribution of the coronary arteries coexisting with an acclerated atherogenesis and progression of lesions. These minor deviations of atherogenic character usually consisted of an increase in external diameter and length of the right coronary artery coexisting with an underdeveloped left circumflex artery or vice versa, or of an increase in external diameter and length of the left anterior descending artery coexisting with an underdeveloped posterior descending artery or vice versa. Some atherogenic minor deviations not only produced a more rapid onset of plaques, but also accelerates the progression of early plaques toward lesions of possible clinical significance.[4] Intimal thickening developed in similar topographic sites of the coronary arterial tree 5 to 15 years earlier in subjects with, than in subjects without, atherogenic deviations from the common type of distribution of the coronary arteries.[5] These results suggest that some of the variability among individuals and populations in the rate of development and progression of coronary atherosclerotic plaques might be associated not only with age, sex, and race, but also with the anatomical branching pattern. Our indirect evidence suggests that the coronary arterial tree geometry may expose an individual at an early age to an increased risk of the onset and progression of atherosclerotic plaques and that this geometry may be inherited.[34]

Not only might the geometry of the coronary arterial tree affect the flow, but the flow may also affect the geometry, since it can modify vessel diameter, angle of branching, and age-related remodelings. Protocols have been implemented to identify these geometric risk factors involved in coronary atherogenesis.[8]

C. Wall Shear Stress

The well-known asymmetry of left and right coronary arteries and the fact that all coronary arteries are highly nonuniform conduits carrying flow patterns which are also nonuniform *predispose to blood instability having an intense shear stress potential.*

The term "shear stress" is attributed to the frictional force acting as a tangential component of the hemodynamic stresses and its intensity can be related to vessel geometry, velocity profile, and fluid viscosity. By definition, this frictional force is equal to the product of the viscosity coefficient (which is a property of the fluid) and the velocity gradient at the interface with the coronary artery wall.

The shear stress represents the component of the force acting parallel to the coronary vessel surface and even when the flow is laminar, the flowing blood tends to exert a *dragging force on the intimal surface* in the direction of flow.

Some observations are consistent with the view that fatty streaks occur in areas where the shearing strain on the vessel is likely to be high, whereas fibrous plaques commonly develop in areas of turbulent flow.[35] A shear-dependent intimal thickening was also revealed. The intimal thickness at sites exposed to high shear stress increases quickly to a modest value, growing slowly thereafter. On the other hand, the thickness at sites exposed to low shears rises more slowly, but after time, reaches higher values.[36] Variations in the intensity

of shear stress were also involved in the occurrence of areas of increased intimal permeability.[37] In these areas, gelatinous lesions may develop secondary to augmented permeation.[38] This development seems to be preceded by small insudates which separate the connective tissue fibers, followed by fragmentation, lysis, and swelling. Such areas may progress to a white sclerotic plaque.[37] In essence, atherosclerotic involvement and intimal thickening are present in regions believed to be high and low in shear.

Whereas the distending arterial blood pressure in the coronary arteries is exerted on the musculo-elastic and ground substance components, the shear stress acts on the endothelium and subendothelial area.

The distending pressure is of the order 100 to 120 mmHg; the force attributed to wall shear stess is only 10 to 50 dynes/cm^2.[39]

The existence in the coronary arterial tree of regions of high and low shear stress has been suggested.[10,40,41]

The human coronary artery wall, with its thickened intima and compact muscular media, seems to be better suited to counteract the distending force of 100 to 120 mmHg of the arterial blood pressure and its pulsatile character, than the much smaller wall shear stress which sets on the endothelial sheet. This assumption is based on the observation that in addition to its pure mechanical effect, the wall shear stress may produce *important changes in the transendothelial passage of plasma components*. Some of these changes might lead to subendothelial accumulation of lipoprotein and fibrinogen macromolecules.

Moreover (theoretically at least), the wall shear stress might create local conditions to stimulate monocyte retention, medial smooth muscle cell migration, intimal cell proliferation, occurrence of lipid-laden cells, intimal insudation and necrosis, lipid deposition, platelet aggregation, and thrombus formation. In essence, it might act as an important atherogenic agent in all age groups and during all stages of the natural history of coronary atherosclerosis.

In certain experimental models it was possible to demonstrate that wall shear stress influences the rate of transport of macromolecules between flowing blood and the arterial wall. At low levels of wall shear stress there is, at best, a very weak shear dependence, but at shear stress levels greater than 50 dyn/cm^2 there appears a much stronger dependence of the transport rate on shear stress.[3] Starting from these and other results, efforts have been made to detect the mechanisms by which means wall shear stress influences endothelial functions, particularly membrane potential and membrane ion transport.

A laminar stream resembles a set of concentric sleeves of fluid. The fluid layer immediately adjacent to the wall moves very slowly because of the viscosity of the fluid. Each succeeding sleeve moves with the velocity of its adjacent mural sleeve, plus an increment imposed by the more central sleeve. Velocities increase from sleeve to sleeve, with the highest velocity in the axial core and the slowest near the endothelium. When turbulence occurs, drag increases with the square of the velocity of the stream. Of particular importance in the coronary arterial tree are the intensified shearing forces which exist at branch sites, since extensive branching characterizes this arterial bed. *At branch sites shearing force is of maximum intensity,* particularly at the point where the rapid axial flow is intercepted by the leading edge of an ostium or the apex of a bifurcation. This intensity varies in the course of the cardiac cycle, as flow velocity and the pressure pulse wave change.

According to certain views, hydrodynamic drag may also act as a specific stimulus determining the vascular caliber.[9] This implies that the endothelium is equipped with receptors or other microstructures that can record and respond to the magnitude of the drag force that impinges up on them. A system was suggested to exist which appears in equilibrium as long as the stress influences endothelial microstructures within physiological limits. Deviations of the drag force from these limits stimulate the cell to initiate feedback mechanisms leading to vasodilation in the presence of intensified shearing forces and vasoconstriction in the presence of very low shearing forces. It may thus be presumed that the change

in flow rate and the associated change in drag induce an immediate physiological adjustment in the smooth muscle cells of the coronary media which are able to relax or to contract. If the changed rate of flow persists over long periods, this physiological adjustment is replaced by an anatomical one, occurring as poststenotic dilatations or, on the contrary, obstructive intimal thickening.

Our present knowledge on the shear stresses that act in the normal coronary arterial tree are very limited. The complex geometry of each major coronary artery with its numerous branching points, the unsteady or pulsatile character of the flow, and the rhythmic compression exerted by the contracting ventricles defies all attempts at simplification by schema and figures.

If an atherogenic role can be attributed to wall shear stress in the onset of fibromuscular plaques, gelatinous lesions, intimal necrotic areas, fatty streaks, incorporated microthrombi, and intramural thrombi, it is the fluid dynamics existing in the coronary arteries of infants, children, and adolescents which requires detailed analysis. In mature adults, the generalized shear stress could well be responsible for the diffuse nature of atherosclerotic involvement.

Analytical studies carried out on experimental models revealed that the tops of the intimal ridges, the junctional areas of endothelial cells, and the nuclear bulge are more exposed to high shear stress.[42] Stress can also be high in regions of constrictions, e.g., in local areas of vasospasm or of vascular compression from adjacent structures. The flow dividers at branch sites appeared in certain works to experience the highest levels of shear stress, frequently approaching several hundred dynes per square centimeter. This may be associated with endothelial cell death, endothelial denudation, and platelet adhesion, all these changes being involved in atherogenesis.[43] Evidence for the involvement of fluid dynamics in the natural history of coronary atherosclerosis centers primarily on the existence of areas of high and low shear stress, usually located proximal or distal to branch mouths. As noted earlier, important differences in wall shear stress intensity are strongly dependent on local geometric factors and flow velocity, which in the coronary arteries varies from 10 to 80 cm/sec.[10]

Variations at the level of blood-endothelium interface are usually related to both spatial and temporal factors. The spatial variations in the intensity of the wall shear stress are mainly due to the anatomical branching pattern of the coronary arteries which is genetically determined and strongly influences regional blood flow distribution. Temporal variations are primarily associated with the cardiac cycle and the compression exerted by the contracting myocardium, this is reflected by the existence of areas of the endothelium with cyclic intensifications or diminutions of the shear stress activity. Of particular importance are those regions which are more or less constantly submitted to high or low shear stress, since only in those areas is it possible to envisage damage related to physical trauma associated with an altered metabolism of the endothelial cells. An imbalance of lipoprotein and fibrinogen transport is likely to occur. In addition, increased or reduced shearing stress might be associated with increased or reduced lateral pressure, tangential tension, turbulence, stretching, vibration, lifting, e.g., acting on an over- or underreactive endothelial sheet.

There is need to emphasize that in certain experimental models even a normal wall shear stress of the order of only 10 dyn/cm^2 was able to affect the transport of albumin, as revealed by the Evans blue technique, albumin uptake increasing with the augmented level of wall shear stress. Additional factors that may influence endothelial permeability are the velocity gradient and viscosity coefficient of blood, both intermingled with the direct mechanical action of wall shear stress. Other factors of variable importance are the level of exposure to shearing stress, the onset of instantaneous peak values of the stress components, and the existence of the rapidly changing directions of stress.

Stress of greater than 1000 dyn/cm^2 has been shown to cause the detachment of endothelial cells, whereas with lower values required to produce endothelial cell denudation, the prevailing change is related to lipoprotein transport into the arterial wall.[44] Among the more

subtle effects exerted by shear stresses of medium intensity on the endothelial cell metabolism is increase in the activity of histidine decarboxylase.[45] There is also an obvious influence of the shear stress on endothelial endocytosis, prostaglandin (PGI_2) production, elaboration of an extracellular matrix, etc.

Also of particular value are the observations which led to the assumption that high laminar flow rates do not normally result in endothelium distortion and disruption, but tend to protect the endothelium and act as antiatherogenic agents.[46] In such vascular segments that have stable flow patterns the endothelial cells align themselves in the direction of wall shear stress along characteristic flow lines.[43,47] The endothelial cells realign themselves in a new pattern if the prevailing shear stress changes in a new stable direction. This dynamic pattern of endothelial cell orientation reflects the average direction of stress exposure. It would be of particular interest to reveal the relationship between wall shear stress and endothelial sheet at the level of the bifurcation area of the left main coronary artery, since the resistance or susceptibility to atherosclerotic involvement in this region of the coronary arterial tree seems to be strongly related to shear stress changes.[48] Starting from the possible role attributed to the shear stress in atherogenesis, there have been several major developments in our understanding of the pathogenesis of atherosclerosis. The complexity of structural and functional changes which may be induced by a hemodynamic signal of a shear stress type has acquired a large theoretical character, being the starting point for a unified view which incorporates both endothelial injury and lipid infiltration hypotheses.[49] According to this unified view, the rate of low density lipoprotein (LDL) infiltration can be influenced not only by elevated LDL levels, but also by endothelial injury produced by hemodynamic stresses (emphasis is placed on the possible importance of transport phenomena within the arterial wall).

In essence, evidence accumulated during the last two decades strongly suggests that wall shear stress is involved in atherogenesis and that its main action is exerted on endothelial cells.[50] Starting from this evidence, some attempts have been made to influence the natural history of coronary atherosclerosis by changing wall shear stress. Thus, the administration of a drag-reducing polymer into cholesterol-fed rabbits inhibited the onset of experimental atherosclerotic lesions.[51] This effect was related to a decrease in vascular shear stress and turbulent flow, both associated with an inhibition of atherosclerotic involvement. These first results suggest potentially fruitful areas for future investigations. It is also possible to influence an atherogenic wall shear stress by means of changes in blood viscosity.[52] The viscoelastic properties of the blood appear to be important determinants of the intensity of shear stress, particularly in areas of secondary flow and near the stagnation points.

Strongly related to the natural history of coronary atherosclerosis are the studies which demonstrated the action of wall shear stress in stenotic areas. If the cross-sectional area of the vessel was reduced by 75 to 80%, the endothelium was abraded within 3 min. This resulted from the significant increase in the mechanical forces exerted by the shearing stress produced by an augmented local blood velocity. Concomitantly, a decrease in lateral pressure on the intima was recorded, whereas distal to the stenosis, the flow tended to become turbulent. Consequent to the endothelial injury produced by this abnormal shear stress, cellular ulcers were visualized, consisting of a focal loss of cell membrane and of a thin layer of underlying cytoplasm. These cellular ulcers were considered to act as "open doors" into the cell; they might also develop spontaneously in areas of disturbed flow and might be regarded as a marker of hemodynamic injury.[42]

In acute studies, endothelial cell integrity remained unchanged until the cells were exposed to excess wall shear stress of about 400 dyn/cm^2.[43] Exposure to this elevated shear stress may lead to endothelial cell necrosis and erosion, whereas the denuded surface became rapidly covered with a layer of fibrin and platelets. This was associated with a massive influx of plasma components into the eroded intima, showing the light microscopic features

of insudation. When a stenosis exists, such changes may promote thrombosis in the post-stenotic areas as a result of turbulence. The rate of platelet adherence to the denuded stenotic areas will determine the rapidity of microthrombi formation and this rapidity appears closely correlated to the intensity of the shear stress. The results recorded in many papers strongly suggest that platelet accretion, microthrombi formation, and plaque development are all closely related to shearing stress associated with turbulence.

D. Hemodynamic Stresses as Related to Coronary Branch Sites

Human coronary arteries are unique among other organ arteries for their nonuniform geometry, the rapid tapering of their caliber, and the abundance of branching areas.

The succession of numerous branching points along the course of the major coronary arteries is a particularly important factor in causing break-up of the flow pattern, a relationship being revealed between the degree of branching and the magnitude of hemodynamic disturbances.

Human coronary arteries are also unique among other organ arteries for their abundance of severe atherosclerotic plaques, often complicated by thrombosis, which develop at branching points.

This particular susceptibility of bifurcations and branch sites for atherosclerotic involvement and especially for the onset of atherosclerotic plaques, has been emphasized in many reports. A study on the location of atherosclerotic plaques within the coronary arterial bed, based on casts obtained by injecting a viscous silicone rubber compound, revealed that 78% of the lesions develop at branch sites.[53] Similarly, 58% of the lesions were present on the lesser and 35% on the greater curvature of the curved segments. A recent investigation of our laboratory showed that coronary atherosclerotic involvement appeared closely related not only to the presence of bifurcations and branchings, but also to the branch mouth diameter. At diameters less than 1 mm of the emerging daughter vessel, the lesional pattern was usually reduced to fibromuscular plaques; at diameters of about 2 mm, fibromuscular, fibrohyaline, and fibronecrotic plaques were detected, whereas at diameters of more than 2 mm, small fatty streaks, gelatinous lesions, and intimal necrotic areas were present.[34] It was also found that the hemodynamic conditions existing in the branch area of the first septal vessel favor microthrombi incorporation and the formation of intramural thrombus. In our investigations, the boundary at the level of the branch site was found to delineate two regions with different aspects of vessel wall remodeling: the age-dependent changes of the major coronary arteries occur as internal elastic membrane degradation, diffuse intimal thickening, and development of longitudinal muscle columns, whereas in the branch vessels such changes did not appear during childhood and adolescence, suggesting a different hemodynamic pattern.[54] If this striking histogenetic difference between parent and daughter vessels of the human coronary arterial bed is discussed from a functional angle, our results offer indirect evidence that the major coronary arteries are submitted, beginning with early fetal life, to intense hemodynamic stresses and that these intense hemodynamic stresses are not operative in the daughter vessels. This may be due to the fact that when the diameter of a branch is half that of the parent vessel or smaller, a significant drop in pressure from the parent to daughter vessel results. Crossing of the longitudinal and circular muscle columns at a right angle near the branch orifice would also lead to a significant drop in pressure from the parent to the daughter vessel.[55] This drop in pressure could become particularly accentuated if a sphincteric mechanism existed at the branching point.[54]

Our studies also revealed heterogeneity in the composition and aggregation patterns of glycosaminoglycans and proteoglycans in coronary artery bifurcations and branching points.[56] In the dilated entrance region of a bifurcation in which blood flow is unidirectional we recorded the presence of the nondigestible fraction of glycosaminoglycans and as individual components, dermatan sulfate and chondroitin sulfates. In the flow divider region we revealed

the presence of dermatan sulfate, chondroitin sulfate, hyaluronic acid, and proteoglycans susceptible to mild alkaline hydrolysis. Finally, in the juxtaostial region, where turbulence is likely to occur, we recorded a prevalence of proteoglycans susceptible to physiological saline solution. The results indicate different patterns of glycosaminoglycan composition and aggregation in each of the three segments of a coronary branch site which probably reflect different adaptations to various types of hemodynamic stresses.

In addition to sphincteric microarchitecture (see Figures 19 and 20) and this heterogeneity in glycosaminoglycan composition and macromolecular organization, our investigations detached in coronary branch sites the presence of intercalated vascular segments.[57] We tentatively termed intercalated vascular segments transintimal large channels, connecting the lumen of the parent vessel with the lumen of the daughter vessel (see Figures 26 to 29). In addition, we revealed in branch sites of the major coronary arteries hyperplastic muscle columns which may encroach upon the branch mouth.[58]

If this peculiar microarchitecture and macromolecular organization of coronary branch sites is related to atherogenesis, the results of our investigations suggest that local hemodynamic and structural patterns exist which favor the onset of atherosclerotic plaques as early lesions. These fibromuscular plaques develop on their own, without a preexisting precursor, often within the branch pads or cushions. *The fibromuscular plaques developed at branch sites progress to fibrohyaline or fibronecrotic plaques and incorporate microthrombi. Many of these advanced plaques remain lipid-free even if they acquire a stenotic character.* On the other hand, the mechanisms of atherogenesis involving insudation, necrosis, and abundant lipid deposition appeared in our material is more effective in nonbranched regions than in adjacent branching points.

Contrary to observations made on human material, in experimental models the branching sites appeared as particularly susceptible to lipid deposition. In the rabbit coronary arteries these experimentally produced lesions surrounded the ostia completely.[59] In cholesterol-fed dogs and swine, lipid deposition generally appeared first at branch points, in the entrance region at the site of intimal pads.[60]

Branch angles and branch diameters are not fixed parameters, they may vary with age, and may be affected by pathological conditions. This would mean that they are sites where the coronary flow might assume unusual characteristics or at least might deviate from its previous pattern. This deviation is reflected in the orientation of endothelial cells and in the occurrence of holes or "craters" in the endothelial surface. At branch sites, a high turnover of endothelial cells was constantly revealed, but when important changes in the flow pattern occurred, the major axes of the ellipsoidal nuclei of endothelial cells tended to align themselves in vector fields in accordance with the associated streamlines in the adjacent blood flow. In the region of the branch mouth, particularly with a diameter greater than 1 mm, the endothelial nuclei tend to be rounded and to exhibit a more or less disordered appearance, suggesting the existence of unsteady flow patterns. When in regions of entrance into orifices the circumferential distension of the hemodynamic stress prevails, a circumferential orientation of the endothelial cells also prevails. In the coronary arteries it may be presumed that the onset of fibromuscular and fibrohyaline plaques at branch sites, as well as the frequent occurrence of platelet thrombi, might be related to regions where flow is likely to be disturbed and separation of stream lines from the vessel wall and formation of vortices may occur. In addition, complex interactions of different flow disturbance are likely to develop at the site of turbulent flow associated with high flow rates. Irregular recirculations are likely to appear, as well as regular flow separations in which flow is separated from the wall by a recirculating dead-water region which can be a slowly moving, almost stagnant fluid.[61] Such flow disturbances, associated with either low or high wall shear conditions were held responsible for the localization of atherosclerotic lesions.[8,43,47]

The tendency to flow separation along the wall of a branch is enhanced as the angle

between the parent and the daughter vessel increases. *An abnormally large branch might represent an undesirable geometric feature*, a geometric risk factor able to give rise to turbulent motion which may act as an atherogenic agent. In our material, this was reflected by a rapid progression of fibromuscular plaques toward advanced fibrohyaline or fibronecrotic plaques. In certain experimental models. the onset of atherosclerotic lesions was correlated with the existence of anticipated regions of local flow separation and/or turbulence produced by branching points. The occurrence of flow separation in pulsatile flow was revealed to be an extremely complex phenomenon, particularly for vessels such as the coronary arteries with their numerous branching points. Flow separation, local areas of high shear stress in close vicinity to areas with low shear stress, as well as areas of flow stagnation and recirculation might all be detected at a branch site. The point of endothelial damage and the adhesion of platelets with liberation of platelet-derived mediators coincides in some models with a migrating separation point.[62] It should be pointed out that arterial pads or cushions develop in flow divider regions in which pronounced stagnation point flow occurs from fetal life. The onset of stagnation point flow also coincides in some studies with the preferential development of early atherosclerotic lesions near bifurcations and branching points. Stagnation point flow in a branching area might also favor microthrombi incorporation in the thickened intima and intramural thrombus formation, as well as microthrombi superposition on preexisting fibromuscular, fibrohyaline, or fibronecrotic plaques. The first step of these complex changes seems to be endothelial injury, followed by platelet deposition and adhesion in the migrating stagnation point flow; an association of the extent of preexisting lesions with the migrating reattachment point was also suggested.[62] In essence, a stagnation point might be a region of deposition of platelets, thrombosis and fibrohyaline plaque development, whereas the reattachment point is a region where blood cells are carried by the flow toward the vessel wall along curved streamlines.[63]

The appearance of vortices also provides favorable conditions for the genesis and growth of platelet aggregates, as well as for the enhanced adhesion of blood cells to the vessel wall in the region of disturbed flow. In the stagnation point flow, the flow vectors are mainly directed toward the arterial wall, whereas at the points of flow separation and flow reattachment frequent collisions occur between blood-borne components. An adequate velocity of plasma components directed toward the endothelial sheet is indispensable, as well as a minimum contact time for subsequent adhesion and transendothelial penetration. When all these conditions exist, the endothelium may be injured, its permeability may be altered, and an important amount of lipoprotein and fibrinogen may accumulate in the subendothelial area; platelet aggregation and adhesion and liberation of platelet components are usually associated with this altered endothelial permeability. A subsequent step includes macrophage retention, smooth muscle cell migration and proliferation in an area of intense or low shear stress, intricated with a stagnation point flow.

These data suggest that both flow separation proximal to the branching point and reattachment of the flow distal to the branching point are of pathogenic significance in atherogenesis.

Certain results would indicate that because of boundary layer separation at branch sites, a barrier to oxygen transport into the thickened intima may occur and could represent an important factor in the complex mechanisms of endothelial injury.[64]

E. Hemodynamic Stresses as Related to Saphenous Vein Graft Changes

The absence of intimal thickening and atherosclerotic involvement in the intramural portion of the left anterior descending artery and the intense susceptibility to both intimal thickening and atherosclerotic involvement of the premural portion of the same vessel would indicate that (1) limitation of distension and/or increased tissue support by the encasing myocardium act as an anti-atherogenic mechanism and (2) distension and/or increased tension act as an

atherogenic mechanism.[65,66] This last effect of distension or increased tension also seems to operate in the onset of atherosclerosis in saphenous vein bypass grafts, leading to progressive disappearance of the patency of the anastomosis.

The natural history of atherosclerosis of an aorto-coronary bypass saphenous veins graft is still poorly understood; an attempt to analyze this natural history suggests the existence of four stages:[67]

1. An early phase of luminal narrowing in the aorto-coronary bypass saphenous vein graft (occlusion at 1 month 8 to 18%, rate per patient 31 to 38%)
2. An intermediate phase of intimal thickening occurring within the first year after operation (5 to 10% of the patent grafts showed at least a 50% segmental luminal narrowing by 12 months)
3. A late phase of occlusion (rate 16 to 26%)
4. A last stage of atherosclerotic involvement, beyond the first year after surgery, the histologic picture being indistinguishable from that of atherosclerotic lesions of the coronary arteries

Atherosclerotic vein graft disease accounts for an important portion of the morbidity after coronary bypass surgery. It may be responsible for the return of angina pectoris, myocardial infarction, and compromised left ventricular function. By its rapid development, atherosclerosis in the vein grafts seems to differ in its natural history from what we consider to be its counterpart in the major coronary arteries. Many pathologists continue to believe that atherosclerotic lesions must appear during childhood to acquire in adulthood the aspect of advanced, complicated plaques. This view can be accepted only as concerns a very limited number of plaques developing in the proximal segment of the major coronary arteries. As concerns the plaques which appear in the intermediate and distal segments of the major coronary arteries and in the proximal segment of the posterior descending, first diagonal, first septal, left and right marginal, sinus node, and atrioventricular node branches, we have demonstrated that they develop during adulthood and that their progression from early to advanced occlusive lesion may require less than 5 years.[6,57] From 100 atherosclerotic plaques present in the coronary arteries of mature adults, only 4 appeared during childhood and only 12 during adolescence. This would mean that 84 of 100 plaques developed during adulthood.

In contrast to arteries in which the circular medial layer is a continuous one, in the saphenous vein (as in other veins) it is made up of interlacing bundles of smooth muscle cells, collagen, and elastic fibers, uncovered by an intimal connective tissue.[68]

It is tempting to presume that if the vein is used as a graft and implanted in the coronary arterial tree, it is submitted to sudden exposure to a high-pulsatile, high-pressure system. This arterialization of the vein leads to important structural changes and is the *sine qua non* factor for atherogenesis.

Platelet deposition occurs in areas of endothelial damage as soon as blood begins to flow through the grafted vein and this might rapidly initiate microthrombi incorporation or development of intramural thrombi. In addition, growth factors from platelets and altered endothelium and from monocytes may initiate and stimulate smooth muscle cell migration in the subendothelial area, followed by intimal thickening and the occurrence of small areas of focal proliferation which may represent an early stage in the onset of fibromuscular plaques.

Grafts which were in place for 1 month usually showed intimal thickening with a vascular mucoid connective tissue in the subendothelial area, some acquiring the light microscopic feature of a gelatinous lesion. After 3 to 9 months, organized thrombi can be revealed, leading to complete occlusion of the lumen, but the prevalent change related to aorto-coronary bypass saphenous vein graft arterialization is a fibrous and diffuse intimal thickening. Foam

cells may also be present in the subendothelial zone as clusters detectable only on light microscopic examination, or as very small spots visible on gross inspection.

Intimal thickening, considered by many pathologists as a proliferative lesion, appears as a natural consequence of subjecting a vein to arterial pressure and to hemodynamic stresses which exist in the coronary arterial tree. This thickened intima is in part similar to that observed in the proximal segment of the major coronary arteries in late fetal life, as well as in the pre- and postnatal periods. On the other hand, a major difference exists: the frequent presence of thrombi in the vein grafts and their very rare occurrence in the normal coronary arteries of fetuses, infants, and children. During the first months after surgery, graft occlusion is due to thrombi, the mechanism of the occclusion being mainly based on the occurrence of such thrombi, particularly at the level of proximal or distal anastomoses. Intimal proliferation of the graft with a stenotic character appear later and may also lead to total occlusion of the lumen approximately 6 months after the operation.[68]

A change in the morphological feature of the thickened intima from prevalent cellular to prevalent fibrotic or hyaline-like tissue can be observed after more than 1 year, whereas typical atherosclerotic plaques require at least 3 years for their development.[69] Under particular conditions, such as excessively high plasma lipid levels, these atherosclerotic plaques may show an accelerated progression and may develop earlier.

Atherosclerosis, identified as a frank plaque, was found in saphenous veins implanted in the coronary arterial bed in more than 70% of the vein grafts, 3 or more years postoperatively. Plaque rupture and thrombosis have also been visualized.[70] Although progression of the lesions with time occurs in all saphenous vein grafts, some risk factors and particularly hyperlipidemia accelerates the evolution of lesions and produces stenotic or occlusive plaques.[71,72] Some forms of hyperlipoproteinemia seem to intensify endothelial injury and mural platelet aggregation, as well as smooth muscle cell proliferation, all leading to an acclerated atherosclerosis in veins exposed to an unusual high and pulsatile arterial pressure. About half of the vein grafts obtained 3 or more years after the operation showed complicated atherosclerotic plaques.[73] Additional changes are also worth mentioning: very thick intimas, adventitial thickening, loss of the inner one third of the media, and loss of elastic tissue.[74] Certain investigators insist on the similarity between the histologic picture of the atherosclerotic involvement of the coronary arteries and saphenous vein grafts implanted in the coronary circulation.

On the other hand, some differences must be also emphasized. Thus, the aspects of platelet adherence, microthrombi formation and incorporation, the onset of intramural thrombi, and intramural thrombi progression toward a fibrohyaline atherosclerotic plaque are by far more frequently seen in saphenous vein grafts implanted in the coronary circulation than in the coronary arteries. The real value of platelet adherence and the consequent release of platelet factors in the initiation of mural and occlusive thrombi and in the onset of the first atherosclerotic plaques is very suggestive in all vein grafts. This may be related to the fact that all vein grafts suffer severe endothelial damage beginning from the moment the vein is obtained from the leg and suddenly exposed to the high pressure system.[75] This might explain why *during the first year thrombogenesis is the prevalent mechanism which may be related to atherogenesis*.

Thrombogenesis then appears intricated with smooth muscle cell migration and proliferation, leading to intimal thickening. In certain regions this proliferation is able to narrow the vein lumen, the early stages of plaque formation often beginning as focal intimal hyperplasia. The late stage dominated by the obstructive character of lesions occurs as intricated thrombogenesis and focal smooth muscle proliferations, with or without platelet adhesion. An occlusion of the graft is possible only as a result of these two intricaed processes, but a superimposed thrombus is also a nonrare event. The fact that by 12 months after surgery up to 10% of the still-patent grafts may show angiographically at least 50% segmental

luminal narrowing,[76] demonstrates the variability of the processes leading to graft occlusion, as well as their persistent aggressive character.

Several factors unique to aorto-coronary bypass saphenous vein graft make the changes induced by hemodynamic stresses more severe in these grafts than in those used in other organs or peripheral circulation cases. Turbulence of flow caused by the anastomosis is very high in the coronary circulation and produces an augmented endothelial damage, platelet adhesion, and fibrin deposition. The vein response to these injuries may occur as progressive organization of subendothelial or mural deposits of fibrin which accumulate as a result of recurrent endothelial damage.[77] Fibrous lesions similar to those seen in bypass grafts were detected in native coronary arteries after endothelial damage from perfusion cannulae or after endarterectomy.[68]

The absence of an adequate experimental model to demonstrate the validity of the thrombogenic theory of atherosclerosis was repeatedly mentioned. On the other hand, aorto-coronary bypass saphenous vein grafts represent an ideal material to follow-up the progressive conversion of intravascular thrombi in atherosclerotic plaques; this atheromatous conversion of thrombi is particularly emphasized in certain works.[78] The origin of concentric fibrous plaques was more clearly related to this conversion into connective tissue of thin laminae of thrombi deposited on the vein intima.[79] The laminated appearance of some of the atherosclerotic plaques developed in bypass grafts, the new vascular channels in the plaques themselves, and the presence of histologically demonstrable fibrin, all lend support to the concept of continued organization of thrombi as the main mechanism of plaque formation.[74]

The immunohistochemistry of these lesions suggest that they arose initially as largely extracellular deposition of fibrinogen-related antigens and apo B-containing lipoproteins in areas of active proliferation of the intima. *These lesions are considered the result of an insudative mechanism in response to arterial blood pressure, associated with thrombi organization.*[80] In addition to unfavorable hemodynamics, endothelial damage, and atherogenic lipoproteins, a factor which may promote the onset of lesions is represented by immune complexes.[81]

Perusal of the literature reveals that the atherogenic effect of hemodynamic stress on the aorto-coronary saphenous vein bypass graft determines a natural history in which fatty streak-fibrous plaque conversion could not as yet be demonstrated, in spite of the rapid development and progression of lesions and in spite of the abundance of available material for study.

F. Hemodynamic Stresses, Arterial Hypertension, and Coronary Atherosclerosis

All types of hemodynamic stresses are intensified in arterial hypertension, leading to an increased susceptibility of the coronary arterial wall to atherosclerotic involvement and to a more rapid progression of early lesions toward stenotic plaques of possible clinical significance.

Hypertension may affect endothelial transport of plasma components and especially of lipoproteins and fibrinogen, alter transintimal and transmedial transport as a result of both intimal and medial thickening and development of thick collagen fibers and influence the geometry of the coronary arterial tree and thus the detailed characteristics of the flow pattern.

The World Health Organization[82] has defined as systolic hypertension more than 160 mmHg, diastolic hypertension more than 95 mmHg, and the range between these two delineations considered to be borderline hypertension. This definition of hypertension as a quantitative rather than qualitative deviation from the norm has been questioned from time to time.

There are three major hypertensive models: renal, deoxycorticosterone salt, and genetic, and conclusions about the atherogenic action in one model may not be appropriate to another model.

The structural alteration associated with elevation of total peripheral resistance would be

the increase in the ratio of wall thickness: vessel lumen radius, so that the latter is reduced even under conditions of maximum vessel dilation.[83] As concerns the functional alteration that prevails, an increase in contractile response and sensitivity to different vasoconstrictor agents was recorded, associated with impairment of the capacity of medial smooth muscle cells to relax.

Studies of subcellular fractions indicate that changes in smooth muscle cell sensitivity may be caused by an increase in cell membrane permeability and an altered handling of calcium by its subcellular sequestering system. The increased sensitivity to vasoconstrictor agents and the decreased responsiveness to dilator agents might be due to alterations in the excitation-contraction coupling and/or electrical properties of smooth muscle cells. These are reflected by changes in:

1. Membrane permeability to ions
2. Activity of ion pumps
3. Transmembrane ion gradient
4. The amount of activator calcium or calcium-regulating contractile proteins
5. The liberation, amount, and degradation of neurotransmitters
6. Drug-receptor interaction, etc.

A particular susceptibility to the action of norepinephrine was detected in regions without denuded endothelium, but with an altered membrane permeability to calcium, and the increased permeability to calcium may account for the augmented myogenic activity. Patients with primary arterial hypertension have increased serum catecholamine levels when compared with normotensive subjects and this level still augments in the presence of psychosocial stress.

There is experimental evidence that hypertension per se, by means of intensified hemodynamic stress and of hormones related to the etiology of hypertension *acts mainly on the endothelial barrier and increases intimal diffusion of plasma components*. This is considered an atherogenic action, but antihypertensive therapy may aggravate the natural history of coronary atherosclerosis; indeed, some antihypertertensive drugs augment total cholesterol, LDL-cholesterol, and triglycerides, whereas β-blockers decrease HDL-cholesterol.[84] This demonstrates that the real link between hypertension and coronary atherosclerosis is poorly understood, as the relationship between these two diseases is very complex.[85] For this reason we have hesitated to reproduce in this book figures, schemas, and diagrams in which these relationships are presented in a too-simplified form.

At least nine main mechanisms are suggested to control arterial pressure:[86] autoregulatory and hemodynamic, mechanical factors, neural factors, catecholamines, renopressor system, hormones, blood volume, sodium interrelations, and depressor agents. In addition, genetic predisposing mechanisms interact with both pressor and depressor factors.

Many investigators consider the increase in artery wall thickness as an adaptive change that is secondary to augmented arterial pressure. In the coronary circulation, the increase in myocardial mass and thickness, the reduction in diastolic filling period, and the augmented small vessel resistance must also be taken into consideration. In certain studies smooth muscle cell proliferation is considered central to the pathology of both hypertension and atherosclerosis. In arterial hypertension this proliferation this involves mainly the media; in atherosclerosis, it is the intima.[87] There is an increase in cell mass in hypertension, and an increase in cell number in atherosclerosis.

In other studies, emphasis is placed in both arterial hypertension and atherosclerosis on endothelial injury, considered a major and common pathogenetic mechanism. Sustained experimental hypertension in dogs is able to produce successive episodes of endothelial injury and repair which may lead to the onset of atherosclerotic plaques. The localization

of denudation areas coincides with that of developing plaque and such areas can be mainly detected in the vicinity of the branching points of the major coronary arteries. The myointimal thickening occurring after endothelial denudation has been reported to be greater in hypertensive than in normotensive animals.[88] In addition, hypertension seems to produce a marked increase in lysosomal hydrolase activity in smooth muscle cells.[89-91] Denudation and atherogenic repair was recorded at branching points in many experimental models in which unusually high hemodynamic stresses were present. These models also showed a greater transfer of LDL-cholesterol into the arterial wall, and with high serum lipoprotein levels, the transfer was further accelerated. An increased intimal permeability to fibrin was also recorded, as well as accumulation of blood monocytes, lymphocytes, and neutrophils in the subendothelial zone, neoformation of fine collagen fibers, increase in the number and contractile state of apparently unaltered endothelial cells, etc. In spite of the complexity of changes produced in experimental models, the real value of these results for a better understanding of the relationships between human arterial hypertension and human coronary atherosclerosis remains controversial.

The capacity of hypertension to increase the severity of coronary atherosclerosis in experimental models was clearly demonstrated more than 3 decades ago.[92] In monkeys, hypertension produced aggravation in the development and severity of the lesions, not only in the major coronary arteries, but also in the small branches.[93] The prevailing change appeared as intimal thickening with luminal narrowing, the atherosclerotic lesions occurring as fibrocellular proliferations associated with the accumulation of abundant ground substance and with an increase in the ratio of collagen to elastin. These altered mechanical properties of the vessel wall may represent one of the multiple atherogenic potentialities of arterial hypertension. They can be also detected in the development of genetically determined hypertension in rats, where a significant augmentation in the arterial wall concentration of collagen is recorded. This might be considered the initial adverse effect of hypertension on the coronary arteries and might coexist with an unaltered lipid metabolism.[94] In these spontaneously hypertensive rats, three evolutive stages were assumed to exist in the natural history of the disease:[95] stage of cell metabolic activation; stage of adaptation, occurring as intimal thickening; and stage of structural and metabolic failure, leading to the onset and progression of atherosclerotic lesions. A particular observation refers to the fact that the correlation between the level of blood pressure and the degree and severity of atherosclerotic involvement is in many experimental models much greater than that between the level of serum cholesterol and the extent of atherosclerotic lesions.[96] Worthy of note is also the observation that hypercholesterolemic rats rendered hypertensive showed an accelerated development of lesions, as well as an accelerated progression toward obstructive plaques able to produce myocardial necrosis.[97] After 3 to 4 months of high fat intake, the coronary arteries of normotensive rats showed only slight lipid deposits, whereas at 1 to 2 months, the coronary arteries of hypertensive rats exhibited well-developed, lipid-rich lesions with superimposed thrombosis.[98]

In man, arterial hypertension could have an adverse effect on coronary arterial wall function and metabolism by increased pulse pressure, altered velocity waveform, increased peak velocity, and increased level of wall shear stress and many other mechanical and hydrodynamic effects.

In man, as a result of hypertension, the coronary arterial bed geometry may be modified; in particular, the angles of the branches may be significantly changed, leading to hemodynamic patterns with more important atherogenic potentialities.

In a study in which the severity of coronary atherosclerosis was determined by measuring the degree of luminal narrowing of the cross-sectional area, this degree was more severe in hypertensive than in normotensive persons.[99] The stenotic index of diastolic hypertensive persons tended to be greater than that of systolic hypertensive persons, particularly in the younger age groups.

In the Framingham study, the relation of antemortem characteristics to cardiovascular findings at necropsy[100] revealed that both systolic and diastolic blood pressure recorded 9 years before death were positively correlated with all degrees of coronary atherosclerosis in woman. In other studies, coronary atherosclerosis was found markedly more severe in the presence of arterial hypertension in both males and females aged 20 to 29 years.[101] In Japanese-American men, coronary atherosclerosis did not appear associated with systolic blood pressure, in contrast to aortic atherosclerosis.[102] In the prospective Oslo study, the post-mortem data indicate that serum cholesterol and blood pressure levels and the number of cigarettes smoked each day could explain only 20% of the variation in raised lesions, with about 80% remaining unexplained.[103] An association was recorded between the occurrence of stenotic plaques which encroached on more than 40% of the luminal diameter and systolic and diastolic blood pressure.[104] A question that cannot be answered with certainty is the influence exerted by these risk factors for coronary heart disease on the coronary arterial wall, on the one hand, and on the myocardium and its conduction system, on the other.

Contrary to the situation of other risk factors for coronary heart disease, the effect of arterial hypertension on the progression of atherosclerotic lesions does not wane with advancing age. At any age in either sex, risk of cardiovascular events has been noted to occur in proportion to the height of the blood pressure. This may explain a continuous exponential increase in risk detected over the whole range of blood pressure. On the average, for every 10 mmHg rise in pressure, there appears to be about a 30% rise in cardiovascular risk. In the Framingham study, approximately 37% of men and 51% of women who died of coronary heart disease had antecedent hypertension; when instances of borderline hypertension are included, the results were 73% and 81%, respectively.[105] Also in the Framingham study, the incidence of coronary heart disease in middle-aged men with blood pressure exceeding 160/95 mmHg was more than five times that of normotensive men with a blood pressure of 140/90 mmHg or less.[106]

It is not possible to limit the adverse effect of arterial hypertension to only an accelerated progression of atherosclerotic plaques toward obstructive lesions. This accelerated progression often coexists with left ventricular hypertrophy, an impairment of the metabolic reserve of the left ventricle, and an increased myocardial consumption per unit weight. With progressive augmentation of the end-diastolic volume or with a progressive decrease in the mass:volume ratio induced by left ventricular enlargement, the metabolic reserve is further impaired by an increase in myocardial oxygen demand. The oxygen supply of the myocardium in arterial hypertension is considered to be influenced by three main factors:[107] (1) the vascular resistance of various segments of the coronary arterial tree, (2) the myocardial extravascular component of coronary resistance, and (3) rheological properties of the blood. Left ventricular coronary blood flow at rest exhibited in certain studies a mean increase of 16% in hypertensive vs. normotensive subjects, whereas the coronary vascular resistance showed an increase of 38%. A mean increase of 50% in coronary perfusion pressure was also recorded, whereas the coronary vascular reserve fell to 42%.[107]

In spite of these complex atherogenic potentialities, arterial hypertension itself, in the absence of other atherogenic risk factors and particularly of certain forms of hyperlipoproteinemia, is not strongly associated to myocardial clinical manifestations and particularly to myocardial infarction. In populations with low LDL-lipoprotein levels, coronary heart disease has a low incidence and the major complications of hypertension relate to cerebral hemorrhage and congestive heart failure.

When cigarette smoking is associated with hypertension, the deleterious effect due to intensified hemodynamic stresses is augmented. An individual who smokes one pack of ciagrettes per day acutely increases his heart rate 20 times in 24 hr because of the direct chronotropic effects of increased catecholamine levels: systolic blood pressure also augments

the rate since direct sympathetic ganglion stimulation and activation of several neural reflexes produce small vessel constriction. These systemic changes increase the workload of the heart and myocardial requirements, leading to an increased coronary blood flow which intensifies all types of hemodynamic stress and particularly the wall shear stress. Moreover, smoking may increase coronary arterial tone at the site of the stenotic plaque, bringing about a decrease in the blood flow to the myocardium.[108] A decrease of coronary reserve was also recorded, which may explain why the threshold to angina also decreases.[109,110] In essence, when cigarette smoking is associated with hypertension, an addition factor able to change coronary vasomotor tone, to produce platelet activation, and to alter endothelial cells is present. This may lead to a more accelerated atherosclerotic involvement than in the presence of hypertension alone.

In summary, alone or associated with other risk factors for coronary heart disease, hypertension is considered a major health problem in economically developed countries and an important atherogenic agent, because it is involved in all stages of the natural history of coronary atherosclerosis.[111-113] A schematic diagram, illustrating some suggested relationships between hypertension and atherosclerosis is presented in Figure 57.[113]

II. SUGGESTED ATHEROGENIC ROLE OF SMOOTH MUSCLE CELL PROLIFERATION

A. Introductory Remarks

Focal proliferations of the intimal smooth muscle cells leading to the onset of fibromuscular plaques represent a main mechanism of atherosclerotic involvement in human beings, domestic, and wild animals — the prototype lesion in spontaneous coronary atherosclerosis.

We would detect, microscopically, the first fibromuscular plaques:

1. In the bifurcation area of the left main coronary artery and the proximal segment of the left anterior descending artery in children aged 6 to 10 years
2. In the proximal segment of the left anterior descending artery, left circumflex artery, and in the proximal or intermediate segment of the right coronary artery of adolescents
3. In the middle portion of the left main coronary artery, proximal segment of the posterior descending, first diagonal and first septal arteries, as well as of the left or right marginal vessels of young adults
4. In the intermediate segments of the left anterior descending and left circumflex arteries and even in the distal segment of the major coronary arteries, as well as in the paraostial region of the sinus node and atrioventricular node vessels of mature adults.

In our material, the onset of fibromuscular plaques in the coronary arterial bed was a basic finding during 4 successive decades of life. In spite of its light microscopic feature of early lesion, the fibromuscular plaque continues to appear not only in young, but also in mature adults in certain segments of the coronary arterial tree. Also noteworthy is the observation that in the intracranial arteries we could reveal the first fibromuscular plaques only in young adults (the vertebral and basilar arteries) and mature adults (the anterior cerebral arteries). It is also in mature adults that we visualized the first fibromuscular plaques in the renal and mesenteric arteries and this would indicate that *during childhood and adolescence fibromuscular plaques develop only in the coronary arteries.*

The successive occurrence of fibromuscular plaques in various arterial beds and in various segments of the same arterial tree leads to the conclusion that atherosclerosis begins as a proliferative disease which has little to do with the so-called experimental atherosclerosis and that fibromuscular plaques develop in the coronary arterial tree during childhood, adolescence, and adulthood. This last observation strongly suggests that the atherogenic agent(s)

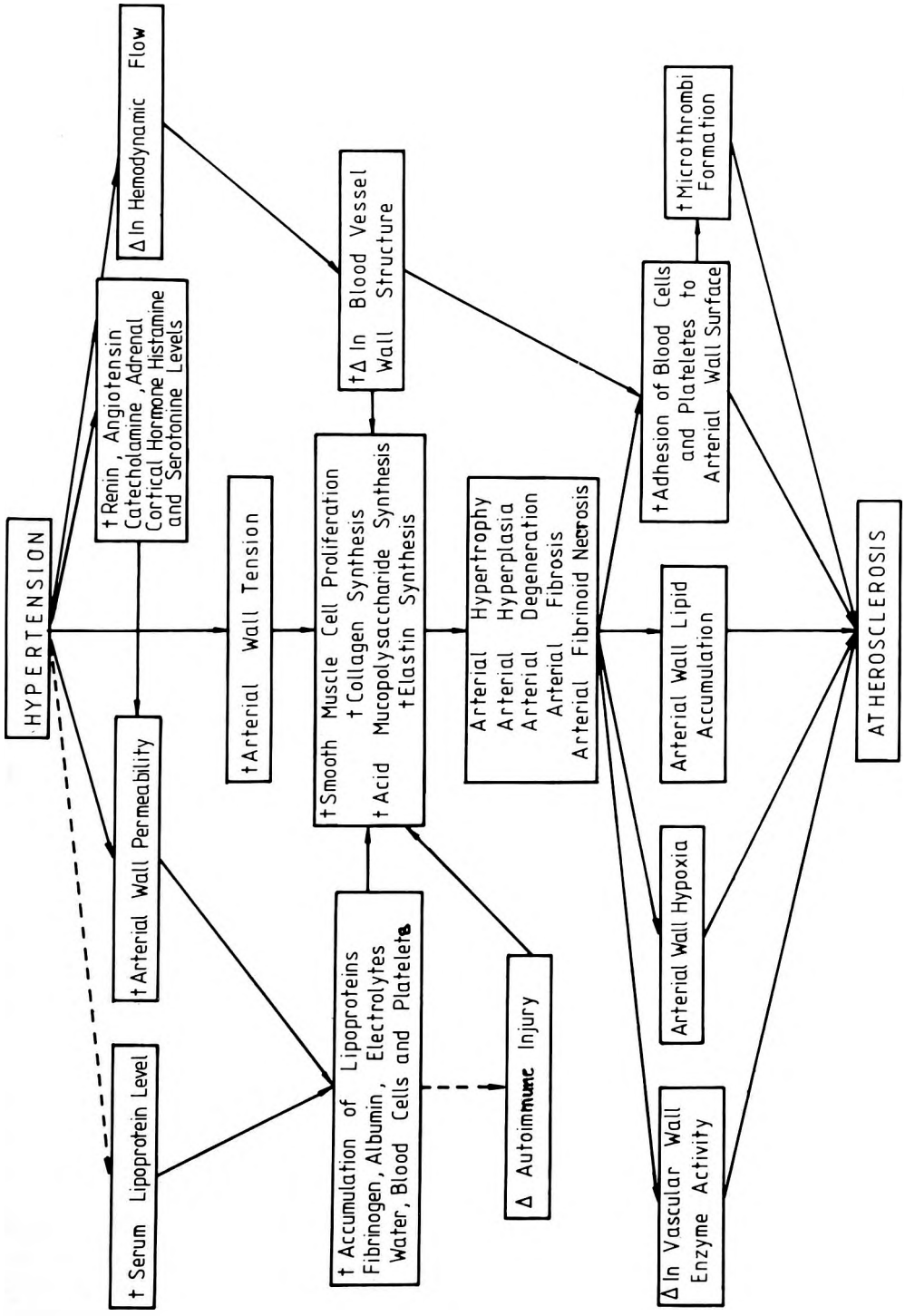


FIGURE 57. A schematic diagram illustrating the possible relationships between hypertension and atherosclerosis. (From Lewis, L. A. and Natto, H. K., *Clin. Chem.*, 24, 2081, 1978. With permission.)

which determine the onset of lesions act continuously on various arterial beds and give rise to atherosclerotic lesions of fibromuscular type in susceptible sites. Except for the bifurcation area of the left main coronary artery and the proximal segment of the left anterior descending artery in which the first fibromuscular plaques were detected during childhood, in all other vessels of the coronary arterial tree, these lesions develop during adolescence, early, and late adulthood.

Smooth muscle cell replication which gives rise to fibromuscular plaques is considered to play a central role not only in atherogenesis, but also in hypertension.[87] These suggested relationships were discussed in the previous section.

In experimental models, cell masses were detected in the thickened intima which showed a constant rate of cell birth and death, involving approximately 40% of the smooth muscle cell population. These cell masses exhibit a focal proliferative response to hyperlipemia and this response occurs in preexisting intimal cell masses present in specific anatomical sites since birth.[114] The further development is considered the initial event of atherogenesis. This view that atherogenesis could be followed up starting from birth might have important implications in the current concepts related to the etiopathogenesis of this disease. The preexistence of "Thomas's intimal cell masses" in the developing intima could be related to the monoclonal character of adult atherosclerotic plaques which will be analyzed in the following pages. On gross inspection, these intimal cell masses cannot be visualized and the respective region is recorded as normal intima, as is the intima with fibromuscular plaques which are not raised lesions and often appear lipid-free.

The smooth muscle cells of the intima have been presented as *multifunctional mesenchymal cells.*[115] The migration of these elements from the media to the intima is considered a prerequisite not only for the onset of fibromuscular plaques, but also for their progression to advanced lesions and the occurrence of a diffuse thickened intima.[116] Once medial cells enter the intima, nonatherosclerotic diffuse intimal thickening may develop and may represent an adequate site for focal proliferation, the most direct evidence resulting from cell kinetic studies.[117,118]

In addition to this migration, relevant findings have been made on changes in the smooth muscle cell cytoskeleton. These changes usually appear during media-intima migration and concern the actin, vimentin, and desmin content per cell, on the one hand, and the expression of actin isoforms, on the other hand.[119] The switch from α-actin to β-actin predominance could represent a reliable marker for replicating smooth muscle cells and in particular of smooth muscle cells involved in the onset of atherosclerotic lesions. Arterial smooth muscle cells having apparently similar ultrastructural features could be separated in populations expressing only vimentin presence, or the presence of both vimentin and desmin. This would indicate that the arterial smooth muscle cells are not homogeneous populations in terms of differentiation and probably of ability to respond to chemotactic stimuli. In the avian arteries, for instance, two distinct types of cells could be delineated, one with the morphologic characteristics of smooth muscle and the second with fibroblastic features.[120] The intermediate filaments of the former cells contain desmin, whereas the intermediate filaments of the latter cells contain vimentin. In the rat arteries, only vimentin-rich cells seem to contribute to the initial intimal smooth muscle cell proliferation induced by an experimental injury.[121]

Worthy of note is the observation that arterial smooth muscle cells could be fractionated into two subpopulations depending upon the capacity of adhesion, structural features, and growth pattern. The high adhesive cells grow as monolayers, while the low-adhesive cells show a marked tendency to multilayered growth, the latter type of cell probably being involved in processes of repair and remodeling of the arterial wall.[122]

These examples suggest that the cell population of the thickened intima is heterogeneous, since to accomplish a multiplicity of functions in the coronary arterial wall, the smooth muscle cell must exist in a wide spectrum of phenotypes.[123-126] At one end of the spectrum

is considered to be the smooth muscle cell whose primary function is contraction. At the other and is the cell actively engaged in the synthesis of the extracellular ground substance and fibers, as well as being actively engaged in division. A smooth muscle cell which migrates from the media to the intima usually modulates from the contractile to the synthetic phenotype before it is capable of division. Consequently, understanding of the mechanisms controlling smooth muscle cell phenotype is considered of particular importance in atherogenesis. A concept was formulated[124] which incorporates features of phenotypic modulation of smooth muscle cells and its relation to proliferation and to synthesis of extracellular material; it also includes the view that the smooth muscle cells which give rise to atherosclerotic plaques have undergone more divisions than their neighbors within the arterial wall. Finally, the hypothesis relating altered smooth muscle metabolic activity to atherogenesis includes features of the response to injury, monoclonal theory, and also suggests how lipid overaccumulation may occur.

During phenotypic modulation the cell may assume a different ultrastructural feature and may present a modified response to an altered environment. Usually, the cytoplasma of this cell contains few myofilaments and large amounts of rough endoplasmic reticulum, free ribosomes, and Golgi elements. These cells are considered to be in the synthetic state of their modulation and prevail during wound repair. In tissue culture, the smooth muscle cells rich in myofilaments and considered in the contractile phenotype do not divide when challenged by serum mitogens, whereas the smooth muscle cells in the synthetic state are susceptible to such mitogens. It is tempting to assume that some smooth muscle cells already in the synthetic phenotype undergoing routine wall maintenance may give rise under particular atherogenic stimuli to proliferative areas; moreover, proliferative areas derived from a single cell may occur and this would account for the monotypic nature of atherosclerotic plaques.[127]

The proliferation of smooth muscle cells of the thickened intima seems to be regulatd, at least in part, by means of negative feedback mechanisms, such as the inhibitory effect exerted on cell division within a population by the population itself.[128] Chemical signals are produced by the cells of the endothelium and by smooth muscle cells which play a major role in the onset and amount of local cell proliferation. Chalones are included in such signals, and are considered to be tissue-specific mitotic inhibitors able to prevent in tissue culture phenotypic modulation from the contractile to the synthetic states.[129] The coronary artery wall, by production of chalone-like substances, may control the phenotype of both medial and intimal smooth muscle cells and, consequently, their capacity to respond to proliferation-stimulating agents, such as those which may come from the blood or from injured endothelium. If the action of proliferation-stimulating agents prevails, many smooth muscle cells modulate from the contractile to the synthetic state. In addition, if the injury is sustained or rapidly repeated, the cells will remain in the synthetic phenotype for a longer period of time and this area of the artery will become particularly prone to the onset of fibromuscular atherosclerotic plaques.

These observations, based on in vitro studies, must be integrated with those which assume that the microarchitecture and cellular composition of the coronary arterial wall is strongly adapted to respond to variations in mechanical stress and strain. As indicated earlier, hemodynamic stress at each site of the coronary arterial tree might be considered the inducer of local changes that adapt vessel wall and caliber to the forces in operation: tensile (stretching), compressive, and shearing forces. The direction, intensity, and duration of these stresses can vary greatly from point to point and the pulsation of pressure waves induces a cyclic rate of change in the strength of each of these forces. The smooth muscle cells must respond to each specific force and provide local adjustment, usually by contraction or relaxation, but also by producing ground substance and fibers, and in some instances by proliferation. It is tempting to presume that stretching of the coronary artery wall will provoke synthesis of collagen macromolecules and formation of fuchsinophil fibers; oscillating tensile forces

will determine a prevalent synthesis of elastin macromolecules and formation of elastic fibers and laminae able to damp the rate of change of tension acting on a certain segment of the artery wall; and an acceleration of tension may lead to important accumulation of myofibrils in smooth muscle cells and the alignment of contractile elements in a manner which neutralizes this injurious effect.[9] It is also tempting to presume that in areas where tangential tensile forces prevail, inextensible collagen fibers develop circumferentially to resist this type of stress. In essence, there seems to exist an essential relationship between mechanical stimuli and elaboration of coronary artery wall macromolecules. It is also important to bear in mind that in the arterial wall many smooth muscle cells are attached to collagen and elastic fibers in positions where they can respond to rapid acceleration of tension and thereby achieve a reduction of tension in the arterial wall; in addition, the amount of smooth muscle cells appears to vary with this acceleration of tension. *The coronary arterial wall can be regarded as a composite multiphase structure in which an abnormal acceleration of tension triggers a countercontraction and/or multiplication of smooth muscle cells, an abnormal rate of change of tension is absorbed by the elastic tissue, and an abnormal tensile force is met by the stretching of collagen fibers.* Proliferation of smooth muscle cells may occur when all conditions in which the preexisting contractile cells, elastin, and collagen fibers become inadequate to counteract hemodynamic stresses; under such conditions, the smooth muscle cells may be directly affected by the abnormal mechanical forces and their response to injury is proliferation.

It is ironic that proliferation of smooth muscle cells of the arterial wall occurs not only as a response to injury produced by abnormal hemodynamic stresses, but also because of the presence of very low hemodynamic stresses. Such very low hemodynamic stresses are produced by a flow at a reduced rate or by experimental ligation of an artery, and in both conditions an intense proliferation appears in the subendothelial area. Similar subendothelial proliferation of smooth muscle cells are observed in the postnatal progressive closure of the ductus arteriosus and in many embryonic vessels. Moreover, in thrombangitis obliterans, the primary disturbance is suggested to be an abnormal sympathetic stimulation which leads to abnormal and prolonged vasoconstriction and a persistently reduced blood flow. This would stimulate smooth muscle cell proliferation in the subendothelial area and the onset of the well-known pathologic features characteristic of this disease. In the coronary arterial tree, all stenotic or obstructive plaques, by reducing flow to the segment beyond the stenosis, may stimulate subendothelial proliferation in the downstream segment of the affected vessel. This may represent the starting point not only of diffuse thickened intimas, but also of diffuse atherosclerotic involvement seen in mature adults and the elderly. Likewise, local subendothelial proliferation of intimal smooth muscle cells may explain plaque enlargement and the new pattern of hemodynamic stresses which develop when the lesion narrows the lumen still further.

All these potential roles of hydrodynamic forces in the adjustment of wall strength and caliber of coronary arteries and coronary branch vessels seem to be very important in the natural history of coronary atherosclerosis. The results furnished by in vitro studies and experimental models frequently overlook the fact that all the proposed pathogenetic mechanisms for the development of atherosclerotic plaques are mediated, at least partly, by mechanical forces associated with blood flow and pressure. Changes observed in atherogenesis are related to an altered metabolic state or reactivity, not only of isolated smooth muscle cells, but of smooth muscle cells when called on to adapt to a variety of different hemodynamic stresses and additional pathologic stimuli.

B. Growth Factor Involvement

More than 2 decades ago, emphasis was placed in certain studies on the capacity of the hyperlipidemic serum from patients with coronary heart disease to exert a growth-stimulating effect on arterial smooth muscle cells.[130]

Of particular value was the demonstration of the existence of specific growth factors, first revealed in platelets. The name of Russel Ross is strongly related to the identification of PDGF (or platelet-derived growth factor), which is able to initiate DNA synthesis in arterial smooth muscle cells.[131,132] The role of PDGF in stimulation of smooth muscle cell movement into the intima and replication within the intima was also demonstrated and related to atherogenesis.[133]

According to the early version of the endothelial injury hypothesis, the initiating event in atherogenesis is removal of the endothelium and subsequent exposure of the thrombogenic components such as collagen fibers of the subendothelial region to platelets which adhere to this altered surface. In a second step, the platelets are activated and undergo degranulation, thereby releasing PDGF, which causes smooth muscle cells to migrate, proliferate, and produce atherosclerotic lesions.[132] This hypothesis is based on the successive occurrence of endothelial denudation and platelet adhesion on the thrombogenic subendothelial area, as they appear in the presence of abnormal hemodynamic stresses, hyperlipidemia, immunologic injuries, etc. This hypothesis has been modified; the endothelium frequently occurs without denudation and monocytes may participate in lesion development.[134] If endothelium is playing a major role in atherogenesis, we should probably not be thinking in terms of crude structural injury allowing plasma components to leak in, but instead in terms of altered surface electrical potential and of all changes leading to altered interaction with components of the hemostatic system. Changes in production of growth factors seem also to be of major importance.[134,135]

Ross' discovery of the existence of PDGF led to the ensuing purification of this factor and identification of its receptors and its genes. PDGF binds to a specific receptor found in smooth muscle cells,[136] but absent in endothelial cells. Human arterial smooth muscle cells contain approximately 40,000 receptors per cell and nonhuman primate smooth muscle cells, approximately 80,000 receptors per cell.[137]

Whereas PDGF appears as a highly cationic hydrophic disulfide-bound protein of approximately 30,000 daltons, the receptor is a cell surface glycoprotein of approximately 170,000 mol wt.[138] The binding of radioiodinated PDGF to PDGF-responsive cells has proven to be a useful method for determination of which cells are likely to be responsive to PDGF.[139]

When PDGF binds to its receptor, it appears to induce a sequence of cellular events beginning with an increased phosphorylation of a membrane protein and culminating in DNA synthesis. This results not only in cellular proliferation, but also in an increase in the microsomal content of smooth muscle cells. In many cells microsomal activation is followed by an increase in the synthesis of collagen, elastin, and various types of glycosaminoglycans. Thus, PDGF is able to trigger cell multiplication and ground substance and fiber formation in arterial intimal connective tissue. This view is supported by the observation that intimal proliferation may be inhibited using antiplatelet agents.[140]

Exposure to PDGF also includes increased binding of LDL to its specific high-affinity receptor. Moreover, augmented degradation of LDL is also stimulated, as well as an increase in cholesterol synthesis and lipid accumulation under hyperlipoproteinemia conditions.

PDGF is not only mitogenic for smooth muscle cells, but is also chemotactic.[141] Its chemotactic activity was related to the frequent presence of inflammatory cells (monocytes and neutrophils) which are attracted to sites of vessel injury where PDGF has been secreted by adherent platelets. Also of interest is the observation that if PDGF binds to the smooth muscle cell receptor it elicits not only an augmented LDL uptake by the respective cell, but also an activation of phospholipase C with the formation of increased arachidonic acid and prostaglandin derivatives, including PGI_2.

The relevance of these experimental data to human coronary atherosclerosis is still obscure. In dogs, for instance, antiplatelet agents are able to produce a marked decrease in the formation of a thickened intima and even in the onset of atherosclerotic lesions,[142] but to date there is no evidence of this important role of antiplatelet agents in human atherogenesis.

PDGF is a protein stored in the α-granules of platelets and secreted during the platelet release reaction. Its mitogenic activity with regard to smooth muscle cells suggests that platelets contribute to hemostasis not only by means of clotting and hemostatic plug formation, but also in a longer term by promoting cell growth factor required for wound repair. It has been calculated that one platelet contains enough PDGF to stimulate hundreds of smooth muscle cells, and small injuries to the endothelium could result, at least theoretically, in a massive proliferative response. Moreover, it has been demonstrated that local vascular injury can cause smooth muscle cell proliferation at a distant vascular site that has been previously deendothelialized.[143] Humoral components may be released into the circulation after vascular injury and may elicit a remote effect on a distant site, since circulating mitogens being able to gain access to smooth muscle cells in all regions where adequate conditions for transintimal passage exist.

The involvement of growth factors in the response of a vessel to injury seems to depend upon at least three main conditions:[139]

1. The capacity of cells adjacent to, or involved in, the damaged area to produce mitogens to which they or their neighbors can respond
2. The variety of growth factors provided by the platelets or other cells, each of these factors being able to stimulate growth under different conditions
3. The physiologic state or local environment which may influence a smooth muscle cell response to different growth factors.

It is not possible to analyze each point in order to demonstrate the complexity of these phenomena. It might be of interest to remember that an autocrine stimulation of growth operating during normal development may be produced by developing smooth muscle cells themselves. This growth factor causes a number of cultured smooth muscle cells to undergo mitosis, its main role being to render cells competent to synthesize DNA. Arterial smooth muscle cells seem able to stimulate their own growth and this could explain why these cells may proliferate in response to an exogenous stimulus for a very long period of time and why they continue to replicate even if the exogenous stimulus has been removed.

An additional known requirement for this cell replication is the availability of somatomedin C (insulin-like growth factor).[144] This additional growth factor can be synthesized by the smooth muscle cells themselves and antibodies to the insulin-like growth factor inhibit cell cycle progression. Growth factors of both PDGF- and insulin-like-type stimulate not only proliferation of smooth msucle cells, but also the synthesis of the intercellular matrix. It is tempting to presume that in experimental models of atherosclerosis the proliferative effect exerted by the LDL fraction of hyperlipidemic serum is related at least partly to the augmented synthesis of the above-mentioned growth factors.[145] Moreover, in experimental atherosclerosis growth factors derived from blood-borne elements may influence smooth muscle cell proliferation and matrix neoformation. The most obvious stimulus seems to be exerted by the growth factor derived from monocyte-macrophages which accumulate in the intima as a result of the administration of a high-fat diet.[146,147] These influences seem to be very complex, since for instance, only platelets may contain transformed cell growth factors, transforming growth factor, a dialyzable factor which stimulates endothelial cell growth factor, and an analog of the epidermal growth factor distinct from PDGF.[148]

The main problem is are all these growth factors produced by platelets, smooth muscle cells, endothelial cells, and monocyte-macrophages able to induce only diffuse intimal thickening not directly related to atherosclerotic involvement, or even the onset of fibromuscular plaques? Since these growth factors are synthesized by many arterial wall cells and blood-borne elements, their presence could explain the rarity of lesion regression during the natural history of coronary atherosclerosis. On the other hand, if each stimulated smooth

muscle cell replicates continuously and never stops its growth, the lumen of the major coronary arteries and of their main branches would be completely occluded in each subject. The fact that only a limited number of male adults show such occlusions demonstrates that all attempts toward simplification may be unrealistic, since while some plaques are still developing, others exhibit an accelerated progression toward stenotic or occlusive lesions, or have stopped growing at various stages of luminal narrowing. The real correspondence of these complex changes seen during the natural history of coronary atherosclerosis with the data furnished by experimental models and with the abundance and variety of growth factors is difficult to establish.

The complexity of the problem is also augmented by the existence of factors that inhibit smooth muscle cell proliferation. Thus, for instance, one of the mitogens secreted by endothelial cells in culture appears to be similar to PDGF, as shown by its capacity to bind to the specific receptors of smooth muscle cell and by the ability of antibodies to PDGF to abolish its mitogenic activity. On the other hand, endothelial cells have been reported to produce factors that inhibit smooth muscle cell proliferation.[149] As endothelium grows over the subendothelial accumulation of smooth muscle cells, it may modify their response in a proliferative or antiproliferative direction. Of particular importance are the relationships between heparin, heparin-like substances, and smooth muscle cell proliferation. It has been known for many years that heparin and other polyanions inhibit serum-dependent growth of cells in culture and in vivo, and that particularly heparin inhibits smooth muscle cell proliferation. The contact between heparin and cell surface seems to be a critical step in the antiproliferative effect. In certain experimental models, heparin-like substances sensitive to heparinases, released by endothelial cells, inhibited the growth of cultured smooth muscle cells induced by PDGF. This suggests a potential role for heparin and related substances as agents able to suppress mitogenic activity and particularly smooth muscle cell proliferation, and this may be regarded as an anti-atherogenic effect.

The amino acid sequence of PDGF is markedly similar to that of the putative gene product of the transforming protein derived from simian sarcoma virus.[150,151] It has been suggested that the normal homolog (c-sis) of the oncogene from simian sarcoma virus (v-sis) is expressed during embryogenesis and development. This gene may be permanently repressed in all cells, but not in the megakaryocytes and platelets which synthesize, secrete, and store PDGF. PDGF might be a normal gene product that becomes restricted to a limited number of cells. A widespread disease such as coronary atherosclerosis, known to have existed at least 3000 years ago, may be considered to be largely dependent on abnormal genes influencing coronary smooth muscle cell metabolism and proliferation for several hundreds of generations. A factor able to initiate DNA synthesis in smooth muscle cells in culture is considered to be a polypeptide homologous to an oncogenic gene sequence seen in human chromosome 22.[150,153] It appears that PDGF is the first growth factor known to be the product of an oncogene, and a viral activation of smooth muscle cells may lead to the expression of c-sis under conditions in which it would not normally be expressed and thus it could participate in neoplastic transformation.[134,150-154] Transformation of cells by a wide range of oncogenic agents may thus be included in the pathogenic mechanisms of atherogenesis, since it appears to activate a cellular gene encoding a growth factor molecule. This growth factor molecule is able to produce not only a rapid proliferation of intimal smooth muscle cells, but also an augmented synthesis of intimal ground substance and fibers.

Marek's disease, a herpesvirus disorder, is associated with atherogenesis in chickens. In the cells of lesions which develop in Marek's disease it was possible to reveal viral mRNA and a viral genetic material which was also detected in arterial smooth muscle cells. Moreover, the presence of herpes simplex genomes in human arterial wall suggests that oncogenic viruses can infect coronary intima and also suggests that this genetic material may be partly or totally responsible for proliferative changes that give rise to fibromuscular plaques. This view is strongly related to the monoclonal origin of atherosclerotic lesions.

C. Monoclonal Proliferation

The eminent pathologist, Dr. Earl P. Benditt, from the University of Washington, opened a new line of theory and research in atherogenesis showing that factors which produce mutational changes in arterial smooth muscle cells may give rise to atherosclerotic plaques.[127,155-157] These mutational changes seem to be analogous to the transformation occurring in benign tumor cells. According to this hypothesis, the atherosclerotic plaques are benign tumors of smooth muscle cells of the artery wall.

The use of the isoenzymes of glucose-6-phosphate dehydrogenase as cellular markers is a technique that was previously employed in defining the clonal characteristics of leiomyomas and other human neoplasms. It allowed the demonstration of the monoclonal origin of fibrous capped plaques which showed an isoenzyme distribution similar to that of leiomyomas.[158] Monoclonal portions were especially delineated in large lesions, predominantly in the center of fibronecrotic plaques, whereas the margins of the lesion often consisted of polyclonal cell populations. The thicker the lesion, the greater the likelihood that monoclonal characteristics would be recorded.[159]

There is at present much interest in the preexistence in apparently normal arterial intima of cell masses very susceptible to proliferate and to give rise to fibromuscular plaques.[114] These scattered Thomas's intimal cell masses may represent the basis for adult lesions originating in preexisting clones of intimal cells and which appear to be of monoclonal origin. It is suggested that alterations of these cells, arising presumably as a result of mutations, may be responsible for the onset of fibromuscular plaques and more advanced coronary atherosclerotic lesions. Environmental premutagens or mutagens could be involved as potential primary etiologic factors in atherogenesis, such as viruses, chemicals, lipid-rich diets, arterial hypertension, tobacco smoking, psychosocial stresses, etc. In fact, urban life, cigarette smoking, and certain dietary elements might be considered of etiopathogenetic significance in both coronary atherosclerosis and cancer. Particularly cigarette smoking is one of those well-documented factors able to augment the risk of both coronary atherosclerosis and lung cancer. Mutagenic hydrocarbons are known to be present in cigarette smoke and can be taken up by blood lipoproteins during passage through the lung and released in the intercellular space of the coronary thickened intima. The burning of cigarettes produces aryl hydrocarbons known to be precarcinogenic; LDLs are the main carrier of these precarcinogens in the arterial wall where they are preferentially taken up by intimal smooth muscle cells.

The monoclonal theory of atherosclerosis also suggests that plaques could be produced by viruses which act as mutagens on arterial smooth muscle cells. The role of viruses in atherogenesis has received and still receives little attention in spite of the demonstration and infection with Marek's disease herpesvirus may lead to occlusive lesions in both hyper- and normocholesterolemic chickens.[160-162] A peculiar observation is that Marek's disease herpesvirus induced lesions in the segments of the arterial tree that were not involved in either untreated control chickens or in chiekens fed cholesterol-supplemented diets, a demonstrative example in this respect being the coronary arterial tree. It is worth pointing out that infection with Marek's disease herpesvirus induced arterial lesions of different character from those produced following cholesterol-rich diets. This difference mainly resides in the prevalence of proliferative changes associated with infiltration of mononuclear cells.[162] In many instances, fatty-proliferative arterial lesions in coronary arteries bore a striking resemblance to human coronary atherosclerosis: collections of foamy-fat containing cells, fatty-hyaline intimal changes, relatively acellular pools of lipid and cholesterol clefts deep in the intima, and fibromuscular caps. Changes in lipid metabolism may contribute to the character and progression of lesions, but the proliferative lesions appeared without dietary cholesterol supplement, being the result of virus infection alone. These experimental results strongly suggest the possibility that persistent viral infections, acting alone or in synergy with other atherogenic factors, may lead to the onset of atherosclerotic lesions.

It is strongly suspected that the biological mechanisms that start spontaneous neoplasm are similar to those which produce atherosclerotic lesions.[163] Both neoplasma and atherosclerosis may occur and develop as autoaggressive disease in persons who have a specific genetic predisposition. In such autoaggressive diseases, including neoplastic, preneoplastic, and non-neoplastic disorders, when a stem cell accumulated its full complement of specific mutation, it propagated a forbidden clone of descendent cells, each of which carries the mutant genes of the progenitor. The autoaggressive attack of forbidden clone cells or of humoral products secreted by such cells on complementary target cells may range from the destruction at one extreme of pathology, to various types of dysfunction, and to neoplastic and malignant transformation at the opposite extreme.[164] According to this concept, atherosclerotic plaques may be regarded as a form of neoplasia.

The amount of agent able to produce malignant lesions, benign lesions, or only tissue hyperplasia is in the coronary arterial wall strongly dependent on the number of lipoprotein particles able to transport it. The greater the level of blood cholesterol or LDL, the greater the concentration of the mutagenic factor that will come into contact with the intimal smooth muscle cells. This relationship was considered similar to that between large bowel cancer and mutagens in bile salts.[165] Tests for mutagens might suggest that some individuals are more prone to produce larger amounts of carcinogens than others in the same environment and this may also be related to an increased susceptibility to atherosclerotic involvement. *Preventive measures should then include attempts to identify mutagens in the diet and in the occupational and nonoccupational environment and then to eliminate these atherogenic agents.* It seems necessary to identify people who are exposed to mutagens which cannot be avoided, people whose life style incorporates the inhalation or ingestion of mutagens (smokers), individuals whose metabolism is deficient in detoxifying enzymes, etc.[165]

As mentioned in Benditt,[157] the monoclonal hypothesis does not immediately simplify the problem of identifying the causes of heart attacks and strokes, but only enriches the information and the ideas that are available to investigators. The existence of atherosclerotic lesions which could be produced by viruses, immune complexes, or polycyclic aromatic hydrocarbon (PAH) carcinogens[166,167] must not be overlooked by those involved in atherosclerosis research. Recent studies revealed, for instance, that an initiation-promotion sequence (modeled after the classical regimens of chemical carcinogenesis) used to elicit epidermal papillomas appeared effective in producing atherosclerosis-like lesions.[168] This would indicate that atherosclerosis may develop in stages analogous to the stepwise development of benign neoplasms. Also of interest is the view that clonal selection with evolution toward a single enzyme phenotype within the lesion could conceivably occur in some kinds of hyperplastic response.[134]

Clonal characteristics were detected in the apparently normal thickened intima.[169] They were also recorded in fatty streak-like lesions and in thrombi. The proportion of samples of apparently normal intima and fatty streaks with monoclonal characteristics was the same, but a bimodal distribution prevailed in fatty streaks. The fact that monoclonal cellular populations are present within apparently normal thickened intima suggest that a subpopulation of cells exist that will undergo clonal proliferation even beginning when the intima still offers the aspect of a normal layer. As concerns fatty streaks, a study of our laboratory showed that from the 2nd through the 7th decades of life, the microscopic counterpart of grossly recorded lesions becomes more complex and conforms less to the largely accepted definition of the fatty streak. Consequently, with aging, we were obliged to reclassify histologically as foam cell-rich plaques, as intimal necrotic areas with clusters of lipid-filled cells, as necrotic plaques with clusters of lipid-filled cells, and as fatty degeneration of intramural thrombi many lesions grossly recorded as "fatty streaks". In the 61- to 70-year-old age group, only 25% of the grossly recorded fatty streaks were true fatty streaks on light microscopic examination. It is thus difficult to assume that a lesion occurring like a

fatty streak is indeed a fatty streak on light microscopic examination.[170] Some lesions which show clonal characteristics between normal intima and fibrous plaques could be foam cell-rich plaques derived from fibromuscular plaques or from mucoid plaques rich in clusters of lipid-filled cells. According to some views, two populations of fatty streaks seem to exist, one destined perhaps by location or risk factors to develop into fibrous plaques and a second which remains as fatty streaks and never goes on to become fibrous plaques.[171] This last conversion is attributed to increased cellularity of the fatty streak. The conversion of fatty streaks in fibrous plaques is also attributed to extravasation of intracellular lipid into extracellular spaces, cellular necrosis, and chronic inflammatory reaction to the extracellular lipid.[172]

Of particular interest are the studies which revealed monotypic smooth muscle cell proliferation in thrombi as a contributing factor to the growth of atherosclerotic plaques.[173] The results showed that 21% of poorly organized (red) thrombi, 78% of moderately organized (pink) thrombi, and 91% of well-organized (white) thrombi showed monoclonal characteristics, similar to those found in fibrous plaques.

When all of the available data are considered together, it is not possible to overlook the view that atherosclerotic lesions may be monoclonal, akin to benign tumors. This must be stressed, since this new fundamental pathogenetic mechanism of atherogenesis requires the development of new rational approaches to prevention: the use of selective inhibitors of specific mutagens. What is necessary is not to reject or neglect this hypothesis because it is not consistent with the exclusive role of lipids in atherogenesis demonstrated by innumerable experimental models; on the contrary, we should concentrate much more research in this interesting field. In agreement with other investigators, we believe that interdisciplinary studies on the connection between developmental biology, oncogenesis, and early events in atherogenesis must become an expected evolution in atherosclerosis research.[87]

D. Smooth Muscle-Endothelial Cell Relationships

An elaborate communication system seems to exist between smooth muscle cells and endothelial cells of the arterial wall, largely influenced by changing conditions. Cells release chemical mediators or communicate via cell-cell interactions in a manner which is considered to be similar to that found in electrical stimulation, nervous, and/or hormonal activities.

There is abundant evidence that the endothelium is first involved in atherogenesis and that this involvement is associated with a variety of biochemical, biophysical, and other types of signals emitted by the injured endothelial cells. The smooth muscle cells and other components of the arterial wall receive these signals and produce a variety of responses, some of which may occur as early atherosclerotic lesions.[174] In experimental models an obligatory role for endothelial cells in the response of vascular smooth muscle cells to various neurochumoral agents was repeatedly recorded. Removal of the endothelium, for instance, abolishes vasodilator responses to acetylcholine, bradykinin, thrombin, and substance P,[175,176] and potentiates vasoconstrictor responses to 5-hydroxytryptamine.[177-179] These results suggest that endothelium injury may play some role in the pathophysiology of abnormal coronary vasodilation or vasoconstriction and even in the onset of coronary vasospasm.

Three forms of endothelial injury are usually detected: denuding injury, increased turnover without denudation, and functional injuries without cell loss or significant ultrastructural alterations.[87] These three forms may overlap, but seem to have different implications for smooth muscle cell-endothelial cell relationships and for the role of these cells in atherogenesis.

The view that atherosclerosis arises as a result of some forms of injury to the endothelial cells was first formulated by Virchow in 1856. This hypothesis was continuously modified during the second half of the 20th century by Ross, Glomset, and Marker; Bjorkerud and Bondjers; Haust; Moore; Joris and Majno; Smith; Schwartz; the Simionescus, and by many other investigators. Many of these hypotheses support the view that some form of injury

may occur to the endothelial cells and this initial event is followed by insudation, smooth muscle cell proliferation, accumulation of monocyte-macrophages, occurrence of lipid-laden cells, and the onset of early atherosclerotic lesions. Moreover, some results suggest that repeated endothelial cell damage may be involved in the progression from early to advanced atherosclerotic lesions and the development of stenotic plaques in the coronary arteries.[180] Also of note is the suggestion that all risk factors for coronary heart disease act by a common mechanism — endothelial hypoxia.[181]

The total population of endothelial cells in the coronary arteries and its relation to the smooth muscle cell population in various vascular segments of the coronary arterial tree is unknown. In the heart the total population of endothelial cells amounted to 3 to 4% of the heart weight and displayed an exceptionally high plasmalemmal surface area.[182]

The mechanisms and factors able to produce endothelial injury can be arbitrarily groups as:[183] (1) mechanical factors, e.g., hemodynamic stresses and arterial hypertension; (2) biochemical changes, e.g., hyperlipidemia, homocysteinemia, hormonal disturbances, carbon monoxide, or nicotine in high levels; (3) viral injury; and (4) immunologic reactions.

The following functions of the endothelium may be altered as a result of an injury (in addition to the classical role of barrier to blood components);

1. Metabolism of vasoactive substances, such as adenine nucleotides, serotonin, bradykinin, and angiotensin
2. Release of inhibitor(s) of platelet functions
3. Synthesis of prostaglandins and removal of circulating prostaglandins
4. Degradation of thromboxane
5. Release of activators of plasminogen and binding of activated coagulation factors (thrombin)
6. Synthesis of surface glycoproteins
7. Activity of muscarinic receptors
8. *Production of factors that inhibit smooth muscle cell proliferation and release of a factor that causes this proliferation*

This very incomplete enumeration demonstrates that endothelial cells not only serve a multiplicity of primary vascular functions, but that they are also highly diversified synthetic cells whose function may markedly influence the response of the coronary arterial wall to atherogenic agents.

Some authors consider atherosclerosis to be "a question of endothelial integrity and growth of smooth muscle".[184] In light of this view, maintenance of endothelial integrity and complex functions appears to be central to arterial wall physiology and implicity to an adequate resistance to atherosclerotic involvement. As long as this integrity and complex functions are preserved, the endothelium:

1. Acts as a physiological barrier separating blood cells and plasma constituents from the subendothelial zone components
2. Provides an antithrombogenic surface
3. Controls the flow of nutrients required for the metabolism of intimal and medial smooth muscle cells
4. Synthesizes macromolecules of the ground substance, basement membrane, glycocalyx, and even for fibrillar structures
5. Produces regulatory factors required by smooth muscle cells for contraction, phenotypic modulation, and replication
6. Elaborates mechanoreceptors that are sensitive to drag

It is tempting to presume that a perturbation of any one of the above-mentioned properties may act as an atherogenic mechanism, the most critical probably being control of smooth muscle cell replication. Cultured endothelial cells have been reported to release growth-promoting activity into culture medium and a hypothesis was advanced that endothelial, cells might produce growth factors in the vicinity of an injury, possibly stimulated by the local formation of thrombin from prothrombin.[139] On the other hand, smooth muscle cells are able to produce an inhibitor of endothelial cell growth.[185] This inhibitor is considered to belong to a class of natural endothelial cell growth inhibitors derived from avascular tissues. Smooth muscle cells would produce at least two factors that may affect endothelial cell growth in different ways.

In a broad sense, a focus of injury to the endothelium represents an assembly of metabolic alterations which may be associated with an assembly of degenerative, destructive, accumulative, and regenerative changes. A damaged endothelium is usually considered unable to offer resistance to hydraulic pressure and to transport of plasma components. Likewise, it is considered unable to adequately cover the underlying subendothelium; this sublayer includes a diversity of organized macromolecules occurring as typical collagen fibers and fibrils, immature fibrils, aggregated elastic macromolecules, basement membrane-like material, and ground substance.

Many experimental studies suggest that injury to the endothelium must be considered the main atherogenic mechanism, a direct relationship being demonstrated between the frequency, magnitude, and duration of endothelial damage, and subsequent onset of atherosclerotic lesions. In general, these experimental studies are based on the production of extensive endothelial damage, followed by extensive exposure of the subendothelial sublayer to the blood flow components and hydrodynamic forces. This leads to a cascade of events in which platelet adhesion, aggregation, and degranulation is frequently mentioned. In addition to activation of the coagulation system, proliferation of intimal smooth muscle cells was recorded. Whether all these complex changes have something to do with human coronary atherogenesis is a question of individual opinion, since no similar features might be detected in post-mortem studies. Only in experimental models can the investigator demonstrate that immediately following injury, platelets are seen adhering to the exposed subendothelium and producing an almost complete covering. Platelet accumulation may lead to the occurrence of small thrombi, followed by shearing off of the protruding platelet masses. Such fragments of platelet-fibrin thrombi might embolize the intramyocardial vessels and might cause ischemic changes,[186] or sudden cardiac death.[187]

Occurrence of thrombi was more rarely detected than the onset of a single thickness platelet layer which persisted as a stabilized structure, intermingled with scattered neutrophiles and macrophage-monocytes. The most dramatic changes take place in the subendothelial region which is invaded by smooth muscle cells migrating from the underlying media and this migration is followed by progressive development of an intimal connective tissue. A striking feature in certain experimental models is the tendency of intimal smooth muscle cells to proliferate into successive sublayers or as nodular proliferations giving rise to lipid-free fibromuscular plaques. When hyperlipidemia exists, these lesions include many lipid-laden cells and acquire the feature of lipid-rich plaques; likewise, an accelerated progression from early to advanced lesions could be recorded. Starting from these experimental data, it is tempting to presume that individuals who do have chronic endothelial injury become susceptible to the onset of fibromuscular plaques in the absence of elevated levels of blood lipids. When these elevated levels exist, the progression of lesions toward stenotic and occlusive plaques of possible clinical significance is accelerated and the respective lesions include a large lipid-rich necrotic center.

Also interesting is that in certain experimental models an inhibition of lesions development following endothelial injury was obtained in animals rendered severely thrombocytopenic.[188]

A further point of interest is that endothelial cells produce and secrete plasminogen activators. In vitro studies revealed that cocultivation of endothelial cells with vascular smooth muscle cells resulted in a significant decrease in plasminogen activator activities secreted by the endothelial cells.[189] The production of a powerful plasminogen activator inhibitor by smooth muscle cells may interfere locally with the fibrin-clearing function of fibrinolysis, which may ultimately result in thrombus formation.

Endothelial injury in experimental models appears to be involved in the onset of micro- and intramural thrombi, fibromuscular plaques, fatty streak-like lesions, and gelatinous lesions and intimal necrotic areas. All factors able to produce an altered transendothelial transport, an altered metabolism of endothelial cells, an altered platelet interaction, and an altered control of growth factors and growth factor inhibitors production may induce early atherosclerotic lesions. To understand the significance of these changes and also the significance of each particular alteration, it is important to know if a great variety of endothelial cells exist, having a miriad of individual functions, or if the endothelium includes a system of cells which play significant roles in the interaction of all cell systems of the organism.[190]

The response to injury hypothesis, in its revised form,[134,184] suggests that injury to the endothelial cells leads to endothelial dysfunction and potentially to endothelial cell-cell separation, endothelial cell-connective tissue separation, and ultimately to endothelial loss, denudation, and exposure of the underlying subendothelium to the flowing blood. This hypothesis includes a range of possible sources of injury to the endothelial cells so that endothelial denudation does not necessarily occur. At least two pathways of atherogenesis are taken into consideration in this revised response to injury hypothesis.[134] The first is based on data obtained in experimentally induced hypercholesterolemia and includes the migration of subendothelial monocytes which become macrophages and engulf lipid to produce fatty streaks, which then progress to fibrous plaques. The second alternative pathway includes release of growth factors leading to smooth muscle cell proliferation and plaque formation. We could not relate the first pathway to observations made in our material, since fatty streaks spare coronary branching points in which fibrous plaques frequently develop and because the great majority of coronary fibrous plaques develop on their own, without any particular precursor. In addition, in human coronary arteries the early atherosclerotic lesions have a very complex light microscopic appearance: fibromuscular plaques, gelatinous lesions, intimal necrotic areas, incorporated microthrombi, intramural thrombi, fatty dots, streaks, or other forms of lipid-filled cell accumulation. The second proposed pathway is far more attractive; we do not know if all fibromuscular plaques which develop in human coronary arteries as focal proliferations of smooth muscle cells are the result of an abnormal production and/or concentration of growth factors. We can only ascertain that in the coronary arteries of children, adolescents, and young adults we have frequently seen focal proliferations of smooth muscle cells occurring in an apparently unaltered vessel, or within a branch pad or cushion or a very thick intima. These focal proliferations give rise to fibromuscular plaques in the absence of hypercholesterolemia or a preexistent fatty streak. This could explain the presence of fibromuscular plaques in the coronary arteries in millions of individuals from Asia, Africa, and South America with serum cholesterol levels of 100 to 150 mg/100 mℓ; it might also explain the presence of advanced plaques in areas spared by fatty streaks. In essence, the view that some kind of injury or abnormality of the endothelial cells seems to be the initial step and essential prerequisite in atherogenesis is largely accepted, but its real significance and importance in human disease is difficult to demonstrate. It is not possible to overlook the fact that some mutagens might stimulate the onset of fibromuscular plaques acting directly on intimal smooth muscle cells, without endothelial injury. It is also necessary to emphasize that injury to the endothelium has been caused experimentally by a variety of traumas without subsequent stimulation of smooth muscle cell replication. A further important feature of lesions induced by repeated endothelial injury which has little to do with

human lesions is the rapid regression or involution when the injury is removed or is no longer present.[188] Likewise, an important observation refers to lipid accumulation in the neointima covered by regenerated endothelium, this accumulation occurring as a secondary phenomenon.[191]

III. SUGGESTED ATHEROGENIC ROLE OF LIPIDS

A. Introductory Remarks

Lipid hypotheses regard atherosclerosis and its natural history as a consequence of an intraarterial overload of lipids (particularly of atherogenic lipoproteins of LDL type) beyond the capacity of intimal cells to handle them effectively.

During the last 2 decades the complexity of the mechanisms leading to VLDL-LDL (VLDL = very low density lipoprotein) conversion and of VLDL-independent LDL formation has become particularly evident.

Based on the results of innumerable experimental models, lipid hypotheses consider that atherogenesis begins with hyperlipidemia, as in cholesterol-fed animals, followed by a loss of the homeostatic balance and the occurrence of a train of events that culminate in the onset of an early atherosclerotic lesion of a fatty streak-like type. These early lesions would progress to advanced forms and the intracellular prevalence of accumulated lipids is replaced by an extracellular one. Many investigators still believe that atherosclerosis is nothing more than a reaction of the artery wall to lipid accumulation, particularly to the irritation produced in the intimal connective tissue by progressive cholesteryl ester deposition.

Starting from the view that the most convincing way to prove that we know what causes a given phenomenon is to produce it at will, a causal link is accepted by many investigators to exist between lipids and atherosclerosis. According to this view, the experimental models of atherosclerosis would represent "the hardest and most direct biological evidence for the lipid infiltration concept that we possess at this time".[192]

In the light of recent findings, it is tempting to presume that *lipids may act as atherogenic agents in a manner which does not require their abnormal intraarterial accumulation,* as seen in experimental models, much more subtle mechanisms being at work, such as:

1. Alterations in the permeability of the endothelium in such a way as to lead to increased entry of atherogenic agents in the intima
2. Direct influence of lipids on the metabolism of some atherogenic lipoproteins, platelets, and coagulation factors
3. The capacity of some lipoproteins to act as mitogens for the intimal smooth muscle cells and as chemotactic agents for monocyte-macrophages from the flowing blood
4. The capacity of some lipoproteins to transport and concentrate in intimal smooth muscle cells products of metabolism, various substances and viruses able to induce monoclonal cell proliferation
5. The capacity of some lipoproteins to give rise to antibodies and to stimulate immune reactions

These are only a limited number of examples to demonstrate the suggested atherogenic roles of lipids; experimental models are only oversimplified versions of these complex pathologic phenomena.

The tendency to oversimplification can be also deduced from the results of many investigations which consider that the interactions of lipoproteins with the arterial wall components and the resultant metabolic changes follow stepwise a similar pattern: the onset of lipid-laden cells and then of fatty streaks which are converted to fibrous plaques. In human coronary arteries, six types of early atherosclerotic lesions were detected in our material:

fibromuscular plaques, gelatinous lesions, intimal necrotic areas, fatty streaks, incorporated microthrombi, and intramural thrombi.

Theoretically, lipids involved in atherogenesis might give rise to fibromuscular plaques, following their action as mitogens on intimal smooth muscle cells susceptible to proliferate; gelatinous lesions and intimal necrotic areas, occurring as a result of focal endothelial alteration allowing massive penetration of plasma components into the intimal connective tissue; incorporated microthrombi and intramural thrombi, as a result of a direct influence on platelets and coagulation factors; and fatty streaks, as a result of abnormal intracellular accumulation of atherogenic lipoproteins.

The tendency to oversimplification is also reflected in the atherogenic role attributed to genetic factors, this role being usually reduced to changes in lipid metabolism. Genetic factors exert their influence on atherogenesis not only by means of atherogenic lipoproteins, but also by changes in coagulation factors, platelet behavior, endothelial and smooth muscle cell metabolism, availability of monocyte-macrophages acting as scavenger cells, anatomical branching pattern of the coronary arteries, etc. These genetic influences are intermingled with those exerted by dietary and environmental factors. Diets high in saturated fat and cholesterol, for instance, could stimulate the production of chylomicrons and VLDL remnants and could lead to impaired catabolism of the remnant lipoproteins and LDL by suppression of apo B and apo E (LDL) receptors.[193] On the other hand, alterations in lipid metabolism could be also produced as a result of specific herpesvirus infection,[194] and this clearly demonstrates the complexity and interrelation of factors and mechanisms involved in atherogenesis.

If only intraarterial lipid accumulation is taken into account, this may be influenced by:[195] (1) the rate of entry, as related to endothelial permeability, plasma concentration, blood pressure, and hemodynamics; (2) rate of egress, which may be influenced by intimal thickness, intimal macromolecular organization and composition, prevalence of the ground substance or fibers, as well as by the molecular weight of the matrix components; (3) rate of destruction, including cell death, severity of hypoxia, leakage of lysosomal enzymes, low pH, and presence of protease inhibitors; and (4) tight binding with ground stance and fiber components. The concentration of atherogenic lipids in the intima is the result of the rates of entry, egress, and destruction, largely influenced by tight binding.[195]

Unfortunately, we still know so little about how lipoproteins enter the artery wall, now they interact with cells, or what the consequences of quantitative or qualitative changes in the plasma lipoproteins would be.[196]

Of note is the observation that the endothelium exhibits the tendency to trap LDL in the intima instead of keeping it out and this raises fundamental questions about the functions of arterial endothelium, since it is able to transport LDL into the intima against a twofold concentration gradient.[197] An additional important and particular observation is that there is a highly significant correlation between increasing intimal cholesterol ester and age, which does not exist between increasing intimal cholesterol and smooth muscle cell proliferation occurring as intimal thickening. This would indicate that in man cholesterol accucmulates primarily as a result of the aging factor, intimal thickening not being the result of cholesterol accumulation as in experimental models.[198] Moreover, this age-related lipid accumulation seems to be similar to that detected in aging tendons, fasciae, synovial membrane, and other tissues, and is not necessarily a step in atherogenesis.

A different opinion maintains that "lipids seem central to the atherosclerotic process beginning in childhood",[199] but we were unable to find in the available literature published papers which demonstrate that the early lesions which appear in children are produced by lipids as in experimental animals submitted to cholesterol-rich diets.

Finally, a very interesting view, consistent with our own findings, is that "even it is not the prime initiator of the lesions, hyperlipemia contributes greatly not only to vessel narrowing, but also to progressive damage to the arterial wall".[200]

A coherent hypothesis regarding the role of lipids in atherogenesis would require not only data on the progressive accumulation of lipoprotein components with in the arterial wall, but also adequate knowledge of:

1. The relative uptake of various types of lipoproteins by the arterial wall cells
2. How this uptake varies with blood lipoprotein levels, lipoprotein pattern, vessel geometry, and prevalent hemodynamic stress
3. The intimate mechanisms by which lipoprotein particles are incorporated and metabolized in endothelial cells, smooth muscle cells, and intimal macrophages
4. The endothelial and subendothelial changes which favor lipoprotein retention or produce their structural modification
5. The mechanisms by which arterial wall cells can shed excess cholesterol or avoid the uptake of lipid products that might be involved in atherogenesis
6. The influence of various lipoproteins and lipoprotein components on platelets, coagulation factors, growth factors, various mitogens, premutagens, and on factors that inhibit smooth muscle cell replication
7. The capacity of lipoproteins themselves to transport and concentrate in intimal smooth muscle cells one or more of these factors which stimulate or inhibit proliferation, and particularly those involved in monoclonal cell proliferation

The lipid hypotheses, based on data derived from experimental, epidemiological, and clinical studies, include the postulate according to which by reducing the level of serum cholesterol in an individual or a population group, a reduction will be obtained in the risk of developing myocardial clinical manifestations induced by coronary atherosclerosis. Indeed, there are many strong arguments relating the level of atherogenic lipoproteins and especially of LDL-cholesterol to the final stage of the natural history of coronary atherosclerosis: (1) some inborn and acquired diseases with hypercholesterolemia are associated with premature myocardial clinical manifestations produced by stenotic or occlusive atherosclerotic plaques; (2) higher serum cholesterol levels can be detected in patients with myocardial clinical manifestations than in the general population and persons with high serum cholesterol levels in prospective studies develop myocardial infarction more often than those with normal serum cholesterol levels; (3) the average serum cholesterol level or dietary fat intake in different countries and populations varies in relation to the mortality from coronary heart disease and decline of this mortality is associated with a decrease in serum cholesterol levels; (4) arterial hypertension, cigarette smoking, and other major risk factors favor myocardial clinical manifestations induced by coronary atherosclerosis when high serum cholesterol levels exist. In man, it was not possible to demonstrate that cholesterol or a particular type of lipoprotein may produce atherosclerotic lesions as in experimental models, but there are innumerable results which show that hypercholesterolemia aggravates the evolution of preexistent lesions toward stenotic and occlusive plaques of possible clinical significance. In some populations and individuals, cholesterol appears as the most important risk factor for coronary heart disease and as a main aggravating agent of the natural history of coronary atherosclerosis. On the other hand, every individual is unique, and cholesterol may be a major risk factor in one case and not in another. The application of mass data to one person is questionable, as is the use of the terms risk reduction and true prevention in a similar sense.

Based on the review of evidence worldwide, it is generally believed that serum total cholesterol values of 140 to 180 mg/100 mℓ are associated with the lowest coronary heart disease rates. Values of 180 to 200 mg/100 mℓ are also associated with a favorable natural history of coronary atherosclerosis. In order to achieve these values for adults, average values of 140 mg/100 mℓ must be maintained in childhood. In our unselected Bucharest population,

a serum cholesterol level of 140 to 160 mg/100 mℓ during childhood and adolescence was associated with the presence of atherosclerotic plaques in the coronary arteries of 2% of children aged 6 to 10 years, 4% of children and juveniles 11 to 15 years old, 12% of adolescents, and 58% of young adults. In certain of these young adults, the atherosclerotic plaques narrowed up to 75% of the coronary lumen. Our data show that with a low serum cholesterol level during childhood and adolescence, many young adults have atherosclerotic plaques in their coronary arteries.[201-203] The fact that serum cholesterol levels of children may be 185 mg/100 mℓ in Wisconsin or 174 mg/100 mℓ in Tecumseh (U.S.), 139 mg/100 dℓ in New Guinea, 130 mg/100 mℓ in Masai Africa, or 100 mg/100 mℓ in Mexico is not an indication that atherosclerotic plaques will develop only in the coronary arteries of children from the U.S. A comparative pathologic study carried out more than 2 decades ago showed that a group of young Asians eating a low-cholesterol diet exhibited a more or less similar extent of coronary atherosclerotic involvement as young American soldiers ingesting a rich cholesterol diet. The lesions in the coronary arteries of the Asian youths were often lipid-free with a prevalence of nonstenotic fibromuscular plaques and fibrohyaline plaques. On the other hand, the coronary arteries of the American soliders exhibited many fibronecrotic plaques with a large necrotic center rich in lipid deposits and a stenotic character.[204] In essence, both Asian and American youths showed coronary atherosclerosis, but the Asians had a serum cholesterol level of only 100 to 120 mg/100 mℓ exhibited a natural history of plaques without progression toward severe lesions of possible clinical significance. The difference between these two groups can be mainly related to the propensity of early atherosclerotic lesions to progress toward advanced forms and this progression appeared clearly associated with a high serum cholesterol level. This may help to explain why in many publications emphasis is placed on the need to reduce serum cholesterol levels in population groups of industrialized countries, better nutritional habits being recommended for all, while at the same time pinpointing special efforts for those at very high risk.

In a publication which reflects the experience of some noted authorities in the field,[205] the following blood cholesterol values are considered for some form of therapy: 20 to 29 years old, >220 mg/100 mℓ (high risk) and >200 mg/100 mℓ (moderate risk); 30 to 39 years old, >240 mg/100 mℓ (high risk) and >220 mg/100 mℓ (moderate risk); and 40+ years old, >260 mg/100 mℓ (high risk) and >240 mg/100 mℓ (moderate risk). The goal of the treatment should be to reduce blood cholesterol to approximately 180 mg/100 mℓ for adults under age 30 and to 200 mg/100 mℓ for those aged 30 or older.

It might be of interest to remember here that White Carneau pigeons, intensely susceptible to both spontaneous and experimental atherosclerosis, have relatively low serum cholesterol and LDL concentrations — approximately ten times lower than that of patients with familial hypercholesterolemia. These relatively low serum LDL concentrations are similar to those of Show Racer pigeons which are resistant to both spontaneous and experimental atherosclerosis. In White Carneau pigeons, the atherosclerotic plaques appear and progress to advanced forms while consuming a cholesterol-free diet and in the absence of elevated serum cholesterol levels.

The observations that in man hypercholesterolemia is not a prerequisite for the onset of coronary atherosclerotic plaques, as it is in experimental models of atherosclerosis, are in agreement with other data offered by comparative pathology. Spontaneous atherosclerosis is often detected in animals with serum cholesterol levels from 40 to 100 mg/100 mℓ. Two decades ago, a study carried out on the pathologic character of atherosclerosis in New World monkeys,[206] revealed that spontaneous fatty streaks and raised lesions appeared with cholesterol levels ranging from 90 to 130 mg/100 mℓ. Lesions could also be produced with diets which did not substantially alter serum cholesterol level.[207]

The irrefutable fact is that in man and in many wild, captive, and domesticated animals, including nonhuman primates, atherosclerotic lesions can occur in the absence of elevated

serum cholesterol levels. This was erroneously used by certain investigators as a proof that the lipid hypotheses are still inadequately substantiated by scientific fact. So far, there is indeed no clear evidence that hypercholesterolemia or any regimen is associated with the onset of atherosclerotic lesions in human coronary arteries; there is also no clear evidence that low serum cholesterol levels or cholesterol-free diets are associated with nononset of coronary atherosclerotic lesions.

The existence of patients with familial hypercholesterolemia is considered by many investigators as an adequate natural experiment to demonstrate that a causal link appears between this familial hypercholesterolemia and coronary atherosclerosis in an atherogenic sense. These patients usually show a galloping natural history of coronary atherosclerosis and not infrequently have myocardial infarction before the age of 10. This was considered the strongest evidence that cholesterol produces atherosclerotic lesions in man as in experimental animals submitted to cholesterol-rich diets.

All cases of familial hypercholesterolemia can be considered to be irrefutable proof that cholesterol, in abnormal amounts, produces a more rapid, severe, and frequent onset of myocardial clinical manifestations related to coronary atherosclerosis. Are these arguments also a proof that lipid is involved in atherogenesis as in experimental animals? In Hurler's disease, a genetic mucopolysaccharidosis, we could reveal a galloping coronary atherosclerosis produced by an abnormal accumulation of glycosaminoglycans and not of lipids. Other pathologists revealed that the proximal segment of the major coronary arteries in patients with Hurler's syndrome is usually significantly narrowed by atherosclerotic plaques.[208] In five of six patients, at least one of the major coronary arteries appeared narrowed 76 to 100% and in four of these five patients all four major coronary arteries were narrowed to this extent.[209] Narrowing of the major coronary arteries is usually diffuse and severe even in children younger than 10 years of age, and in certain papers this narrowing was described to be similar to that of adult patients with fatal myocardial infarction. Thus, a genetic mucopolysaccharidosis characterized by extensive and nearly universal deposition of dermatan and heparan sulfate (due to the absence of a lysosomal enzyme, α-iduronidase) is able to produce lipid-free stenotic lesions in the coronary arteries of children of similar severity to that seen in familial hypercholesterolemia. If we accept the view that a condition which produces fulminant coronary atherosclerosis must be considered of etiologic significance, it is tempting to add not only accumulation of LDL, but also dermatan and heparan sulfates.

Moreover, Kawasaki disease, a clinical syndrome which appears in infants and children with high fever, swelling of lymph nodes, and characteristic mucocutaneous involvement, also produces a galloping natural history of coronary atherosclerosis followed by myocardial clinical manifestations.[210] A rapid development of atherosclerotic lesions was also obtained after administration of homocysteine,[211] a herpesvirus,[160] or following immunologic injuries.[212]

Theoretically, lipid hypotheses are very attractive and could explain why many well-known scientists appeared to be intellectually and emotionally involved regarding the atherogenic role of lipids and the presence of lipids as a hallmark of atherosclerotic lesions.

For more than 50 years, this atherogenic role was mianly attributed to cholesterol, one of the most intensely studied subjects in science, with a remarkable concentration of scientific talent and fiscal resources. Cholesterol is also the most highly decorated molecule in biology, 13 Nobel Prizes being awarded to scientists devoted the major part of their career to cholesterol.

More than 93% of the body cholesterol is located in cells where it performs vital, metabolic, and structural functions. Its role in the cell cycle is not entirely clear, being mainly required for membrane formation. Only about 7% of the body cholesterol circulates in plasma and this is the fraction involved in atherogenesis by means of intraarterial accumulation of cholesterol-rich lipoproteins. All the cholesterol in plasma is packaged with lipoprotein particles, two thirds in LDL, and it is considered able to produce smooth muscle cell

proliferation, endothelial injury, platelet aggregation, and other changes of atherogenic character. As mentioned earlier, the problem of lipid and particularly of cholesterol accumulation within the arterial wall can be viewed not only as a result of elevated circulating LDL levels, but as the summation of many factors, each with its own effect on uptake, synthesis, transport, and degradation.

The average serum cholesterol levels in many male adults 40 to 49 years old in industrialized countries, such as the U.S., could be more than 250 mg/100 mℓ, whereas in other industrialized countries, such as Japan, only 170 mg/100 mℓ. This high serum cholesterol level in the U.S. was related both to a high caloric diet rich in saturated fat and cholesterol and to the augmented incidence of coronary heart disease. On the other hand, the low serum cholesterol level in Japan was associated with a diet usually low in saturated fat and cholesterol and also to a low incidence of coronary heart disease. Japanese in the U.S. show an increased incidence of coronary heart disease when they alter their dietary regimen to approximate those of their adopted country after a period of about 2 decades. Asian and Yemenite Jews which adopt the Western way of life in modern Israel show an unexpected increase in the incidence of myocardial clinical manifestations induced by coronary atherosclerosis. As concerns the migrants moving from high- to low-risk populations, they often preserve the incidence of coronary heart disease existing in their country of origin.

There are, in the available literature, innumerable examples which demonstrate that high total cholesterol level and high serum LDL-cholesterol level aggravate the natural history of coronary atherosclerosis. On the other hand, human coronary atherosclerotic lesions are not produced by cholesterol-rich diets as in experimental animals and hypercholesterolemia is not a prerequisite in human atherogenesis. This is why the diagrams, schema, and figures including such a view are not reproduced in this book, since they may include false premises or preconceived ideas presented as well-established facts. On the other hand, the recognition that some patients with premature myocardial clinical manifestations induced by coronary atherosclerosis have a lipid abnormality (which is transmissible to their offspring) yet treatable by diet and drug, imposes a responsibility upon all practicing physicians. That hypercholesterolemia and other abnormalities of lipid metabolism do not induce atherosclerotic lesions in human coronary arteries, do not cause all progression from early to advanced lesions, and do not cause all myocardial clinical manifestations induced by coronary atherosclerosis, in no way invalidates the importance of cholesterol and of lipid abnormalities in the natural history of coronary atherosclerosis. The Coronary Primary Prevention Trial of the NHLBI (Lipid Research Clinics Program)[213,214] has provided conclusive evidence that lowering cholesterol and LDL will decrease coronary heart disease risk, defined as the sum of nonfatal and/or fatal myocardial infarctions. In addition, the results of other trials demonstrate that if serum cholesterol values decrease by more than 15% in hypercholesterolemic patients, the morbidity and mortality rates significantly decrease. These and many other examples strongly suggest that socioeconomic development has influenced the natural history of coronary atherosclerosis. An accelerated progression of lesions toward advanced plaques of stenotic or even occlusive character appeared associated with this socioeconomic development. The available evidence would indicate that hypercholesterolemia is the most important risk factor for myocardial infarction, whereas its role in the pathogenetic mechanisms of sudden cardiac death could not be convincingly demonstrated.

In essence, the role of lipids as a main risk factor for coronary heart disease is a well-established fact, whereas the role of lipids in the onset of human atherosclerotic lesions remains questionable. Studies carried out on saphenous vein aorto-coronary bypass grafts show that many early atherosclerotic lesions occur as proliferative changes and their onset may be prevented with antiplatelet agents.[215] Lipid accumulation does not usually result unless patients have hyperlipoproteinemia.[216]

A second conclusion of these introductory remarks is that today no investigator can

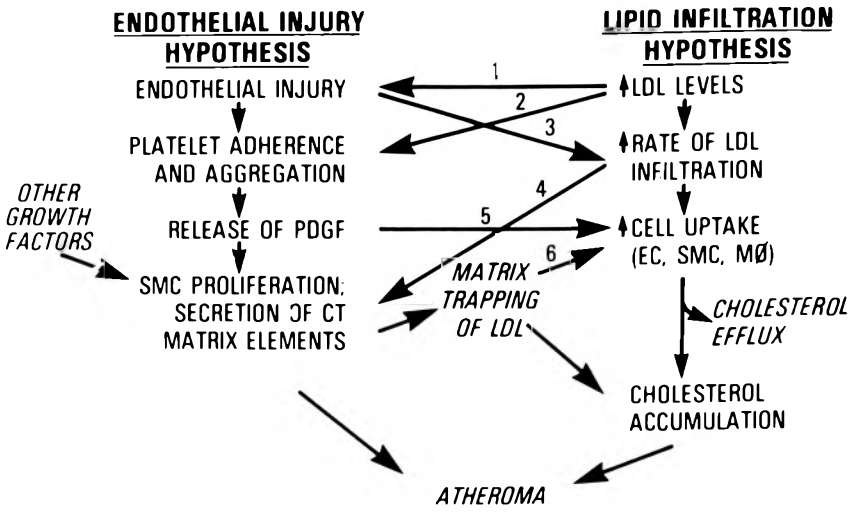


FIGURE 58. The probable interactions between the two major pathogenetic mechanisms proposed for atherogenesis. A proposed unified hypothesis linking the endothelial injury and the lipid infiltration schools. (From Steinberg, D., *Arteriosclerosis*, 3, 283, 1983. With permission).

maintain, with the authority of scientific facts, that a disturbed lipid metabolism or a high serum cholesterol level is a prerequisite for the onset of atherosclerotic lesions in human coronary arteries.

B. Lipid Accumulation as Related to Endothelial Injury

Many data related to the physiology and pathophysiology of the endothelium and especially on its suggested role in atherogenesis have already been mentioned in this chapter.

A suggestion was advanced that the biochemical mechanisms in atherogenesis can be divided into two ongoing processes that frequently parallel and sometimes interlink with each other: (1) the platelet-endothelium interrelation and interaction and the resultant proliferation of smooth muscle cells and formation of intimal matrix and (2) lipid accumulation in the developing plaques.[216] "The so-called lipid infiltration school and the so-called endothelial injury school are by no means virtually exclusive and independent."[196] Figure 58 tries to synthesize this view.[49]

Endothelial cells seem to be deeply involved in lipid metabolism since they have receptors for LDL, VLDL, and chylomicrons. In certain experimental models, LDL receptors bind particles but do not internalize them, the result being a release of a modified LDL which is subsequently taken up by macrophages.[217] As concerns the VLDL receptors and those for chylomicrons, these triglyceride-rich lipoprotein receptors bind particles at the endothelial cell surface, where both VLDL and chylomicrons are submitted to the action of lipoprotein lipase which is connected to the endothelium by a heparin-like material. Whereas endothelial cells release a modified LDL, lipoprotein lipase removes triglycerides and fatty acids from the VLDL and chylomicrons and release remnants; concomitantly, a transfer of C peptides from the VLDL to HDL particles takes place.

The above data show that the term endothelium refers to cells with various prevalences for the synthesis, metabolism, binding, uptake, release, modification, and degradation of plasma components involved in the maintenance of hemostatic equilibrium. Endothelium may also act as a mechanical and oxygen sensor and as a modulator of vascular tone and smooth muscle cell proliferation.

To accomplish these complex functions the role of membrane fluidity is overwhelming, since vesicular transport is very intense, occurring as import (endocytosis), export (exocytosis), and interorganelle transport.

The endothelial cells include many cytoplasmic vacuoles with a wide variety of shapes, sizes, and functions; they are usually encapsulated in a limiting membrane which stems from the organelle of origin by a budding process. An important quantity of material available for membrane components is necessary in each endothelial cell and particularly in those which may internalize abnormal amounts of plasma components. Such cells may internalize the equivalent of twice its entire surface and this may explain why the demand for membrane components is very high. Synthesis alone cannot often match this flux rate and recycling of the various membranes seems to act as an additional mechanism.

Under certain experimental conditions, LDL particles are toxic to the endothelial cells in culture and this injury can be partly prevented by the antiatherogenic lipoprotein HDL. Also in certain experimental conditions it is possible to reveal that elevated LDL concentrations may not only increase the rate of passage through an apparently unaltered endothelial barrier, but may create breaks in that barrier. This injury may open the way for entrance of these lipoproteins into the subendothelial space, and in such areas platelets adhere, aggregate, and release stored material.

More important for human atherogenesis is the observation that when abnormal cholesterol values exist in blood plasma this seems to have a negative influence on the capacity of endothelial cells to maintain the structure and function of each organelle, in accordance with cell physiology. In particular experimental conditions, high cholesterol levels may influence the membrane lipid composition of endothelial cells and may affect its fluidity to the point of occurrence of an injury.

It is largely accepted that cholesterol can regulate the fluidity of both phospholipids and proteins of biological membranes and that in the presence of too much cholesterol, this fluidity is altered and the endothelial cell metabolism is affected. When placed in a milieu containing an increased cholesterol concentration, the cell membrane may maintain fluidity up to a point by increasing unsaturation of the phospholipids. When this adaptation fails, the membrane accomodates to the increased cholesterol by means of cell proliferation. This last mechanism could be related to the capacity of elevated levels of serum cholesterol to promote smooth muscle cell proliferation in tissue culture. In addition, excess cholesterol incorporated in the membrane would decrease the fluidity and hamper cell metabolic activities.[218,219] Of particular importance seems to be membrane changes induced by excess cholesterol which alters calcium transport. This alteration is followed by increased endo- and transcytosis and may lead to cell death. By means of changes in membrane permeability cholesterol may influence the influx of calcium into the endothelial cells and the rate of this influx may appear as a critical factor in atherogenesis. According to certain views, all major risk factors for coronary heart disease influence the development and progression of atherosclerotic lesions by mechanisms which include variations in calcium levels.[220]

Two classes of calcium channels are involved in cell physiology, one potential-dependent and the other receptor-operated; the endothelial cells possess only receptor-operated channels, whereas smooth muscle cells possess both types. The receptor-operated channels of endothelial cells are influenced by chemotactic substances, growth factors, hormones, neurotransmitters, immunologic injuries, eicosanoids, and many other factors and mechanisms, the net result usually being an elevation of the cytoplasmic concentration of calcium. This may be followed by smooth muscle cell and monocyte migration in the subendothelial area.

Changes in membrane fluidity induced by cholesterol might be the main cause of inhibition of the activity of Na^+-K^+ ATPase and might create local conditions which lead to a decrease in the intracellular level of cAMP. This stimulates cell proliferation and may be related to the influence exerted by hypercholesterolemia on endothelial healing, particularly the so-called reendothelialization. In certain experimental models hypercholesterolemia did not delay or accelerate endothelial healing, but the monocyte-macrophage adhered to and penetrated regenerated endothelium only in hypercholesterolemic animals; it was also only in

hypercholesterolemic animals that an increase occurred in the number of cells within the intima and accumulation of lipid in areas of endothelial regeneration.[221]

Also of note is the observation that endothelial cell membrane changes induced by excess cholesterol may hinder the capacity of the endothelium to act as a short-term storage or buffer compartment for control of the level of heparin in the circulation. Excess cholesterol can also modify the capacity of endothelial cells to store adenosine; because of its antiaggregation properties, this capacity may contribute to the antithrombogenicity of the endothelial surface.[222]

Changes in endothelial membrane fluidity induced by excess cholesterol may also influence endothelial participation in the metabolism of vasoactive substances, such as bradykinin, serotonin, norepinephrine, etc.; similarly, the capacity of endothelial cells to release PGI_2 may be altered, a relation being observed between the production of prostaglandins and the susceptibility to atherosclerosis. Particularly, the capacity of endothelial cells to release adequate amounts of PGI_2 is considered of importance in the maintenance of the hemostatic balance, especially the ratio of PGI_2 to thromboxane A_2; LDLs have been reported to reduce endothelial PGI_2 synthesis.[223]

The relationships between cholesterol and endothelial membrane fluidity cannot be overlooked in studies on coronary atherogenesis; on the other hand, their real value in human disease is difficult to delineate. An additional source of endothelial injury related to the atherogenic role of lipids seems to be represented by free radicals. Lipid peroxidation and, especially, free radicals derived from oxygen generated in the univalent pathway of reduction of molecular oxygen to water may alter endothelial cells and produce severe dysfunction. A defense mechanism against an increased level of free radicals in the arterial intima seems to exist, reflected by an augmented activity of superoxide dismutase. The increase lipid peroxide content of the LDL fraction is also considered able to inhibit the generation of antiaggregatory factors produced by endothelial cells.[224,225]

It is tempting to presume that alterations in membrane fluidity of the endothelial injuries produced by free radicals may reduce synthesis of fibronectin, collagen, apo A-I and apo-A-II apoproteins of high density lipoprotein (HDL)[226] which participate in the fibrinolytic process,[227] may reduce the release of relaxing factor for medial smooth muscle cells,[228] and may influence heparin metabolism as well as the metabolism of many other substances and active agents. Since excess cholesterol is considered able to modify the fluidity of endothelial cell membranes, it might be of interest to remember that a variety of substances and functional groups have been identified to have receptors on endothelial cells: vasomotor agents, clotting factors, growth factors, glycosaminoglycans, hormones and neurotransmitters, plasma lipoprotein particles, and products of the immune system. In some subjects the role of lipids in atherogenesis may be not related to their intraarterial accumulation to give rise to fatty streak-like lesions, but to their influence which enhances procoagulant activities of endothelial cells; variations in production of plasminogen activator, plasminogen activator inhibitor, factor V, thrombospondin, and Von Willebrandt's factor, may all be related to atherogenesis.[229,230] Unaltered endothelial cells have the ability to release an enzyme that binds to and locally catalyzes the lysis of fibrin and this modulation may be critical in atherogenesis.

LDL particles with a mol wt of 2.4×10^6 daltons seem to cross the intact endothelium at a very slow rate, but localized endothelium injury would allow abnormal amounts of LDL to leak into the subendothelial space. Measurement of the flux of labeled protein into this space showed that the relative concentrations are directly related to molecular weight and that the concentration of LDL in this location is greater than the concentration in the subject's own plasma.

The virtually linear relation between relative retention and molecular weight found for a range of plasma protein is difficult to reconcile with the concept of specific bind-

ing.[197,231-233] The relation with molecular weight suggests molecular sieving and the steady-state concentration might reflect vesicular transport of whole plasma across the endothelial cell into the subendothelial space. This might be followed by egress of individual molecules by diffusion at rates that are inversely related to molecular weight. According to these results, increased LDL concentrations in the interstitial fluid of the subendothelial area could not result from crude endothelial denudation; the large LDL macromolecule appears to be trapped between the endothelial and subendothelial areas. This macromolecule preserves its freely diffusible form and is not coupled by specific binding with ground substance or fiber components. All changes associated with a greater fluid retention and expansion of the interstitial fluid space may act as atherogenic agents. All changes which hinder the passage of LDL back into the plasma down to the concentration gradient may all act in this sense.[135] The endothelium seems to provide a barrier not only to the penetration of atherogenic lipoproteins into the arterial wall, but also to the outward movement of LDL. This leads to a delivery of excess sterol to the subendothelial space and not back into the plasma, which could explain why the concentration of LDL in the subendothelial space has been found sometimes to be very high.[231-233] In the normal intima the interstitial fluid contains twice the plasma concentration of LDL and at this high concentration the smooth muscle cells neither proliferate, nor fill with fat.[135]

Under certain experimental conditions, the transfer of rabbit LDLs, as evaluated from the transport of labeled serum cholesteryl esters, was approximately 30 times greater in areas devoid of endothelium than in endothelium-covered regions. On the other hand, in the presence of an endothelial barrier, the transfer of rabbit LDLs varies, with a smaller transfer in low injury and a larger one in high-injury regions.[234,235] In experimental hyperlipemia, the rate of transfer from low injury regions of the arterial endothelium may increase approximately ten times and for high injury regions approximately 100 times. This shows that moderate differences in permeability to LDL displayed by severely and less severely injured endothelium are amplified at higher serum cholesterol levels. Some results of these experimental studies may lead to the conclusion that the peculiarities of the endothelium may be as important as lipoprotein abnormalities in atherogenesis.[235] Of particular interest are the investigations which showed that repeated endothelial injury may cause lipid-rich lesions even in animals in a normal diet. Moreover, in severely thrombocytopenic animals, these lesions do not form or are markedly inhibited. The occurrence of lipid in some experimental designs appeared related to continued or repeated thrombus formation. Lipid accumulation was detected in areas where endothelium was repeatedly removed and regrown. In these experiments repeated deposition of thrombus-like material may bring about changes in the metabolism of the neointima which favor lipid accumulation.[236-239]

An intriguing problem is the existence of LDL modified by endothelial cells during transcellular passage and which is taken up by the same receptor that recognizes and takes up acetylated LDL. Since LDL has to traverse the endothelium to gain entrance to the subendothelial space, it is quite possible that this transformation may play a certain role in the mechanism of lipid accumulation. Modified forms of LDL, such as those which pass through the endothelial cells, are recognized by receptors that do not recognize native LDL particles. These peculiar receptors are mainly present on the surface of macrophages and the avid uptake of these modified LDL particles may be followed by the occurrence of lipid-filled cells and fatty streak-like lesions. Monocyte-derived macrophages which are present in the arterial intima have phagocytic properties and also many receptors. One of these receptors binds LDL modified following transendothelial passage and this type of receptor seems to be unsaturated, but continues to pick up modified LDL until the cell is entirely filled with lipid and acquires the light microscopic aspect of a foam cell. This pathway is called the "scavenger pathway".[240]

A new line of research and thought was opened by the investigations which revealed a

characteristic appearance and continuous deposition of lipid material organized as small vesicles formed by phospholipid lamellae rich in unesterified cholesterol. These vesicles with a diameter from 100 to 300 nm are tentatively called extracellular liposomes.[241] These changes are associated with an apparently intact endothelium and no platelet intervention was observed. The results suggest that in the hypercholesterolemic rabbit, lipid deposition in the subendothelial region does not require endothelial injury; the results also show that this deposition begins with the formation of an extracellular pool organized primarily in uni- and multilamellar vesicles rich in unesterified cholesterol. After the appearance of monocyte-derived macrophages, followed by cholesterol uptake and esterification, deposits of intracellular esterified cholesterol may occur, leading to the onset of fatty streak-like lesions.

The subendothelial accumulation of monocyte-macrophages seems to reflect the endothelial release of factors that signal the cells to enter the intima and remove excess cholesterol. Such chemotactic signals may be related to the migration of medial smooth muscle cells and to the retention of blood monocyte-macrophages, both types of cells being able to accumulate lipid and to give rise to fatty streak-like lesions. When macrophages are stimulated, they also release mitogenic factors and if the stimulus is of immunologic origin, this may provide a link between immunologic injury and atherogenesis.[242-247]

Knowledge of transendothelial transport of plasma constituents occurring via different pathways has been mainly advanced by the eminent electron microscopic studies of Palade[248-250] and also by those carried out by the Simionescus and Palade.[251-255]

Transport routes for macromolecules have been studied with probes of various diameters that can be visualized in the electron microscope. One might assume that plasmalemmal vesicles cycle back and forth between endothelial luminal and subendothelial surfaces by Brownian motion, equilibrating their contents with the fluids on both sides, a mechanism which does not appear adequate for the massive reverse transport of LDL macromolecules back into the blood flow.

Most endothelial cells contain large numbers of plasmalemmal vesicles with a diameter of about 70 nm; some appear to fuse with the plasmalemma and open to the luminal or subendothelial surface of the endothelial cells and chains of vesicles may fuse to form channels. Fluid phase endocytosis is considered the main process related to the passage of plasma components into the subendothelial space in the great majority of experimental models. Most available evidence suggests that LDL does not penetrate between endothelial cells, but that the flux takes place through the cells, i.e., by transcellular vesicular transport.[256] This vesicular transport of LDL could be via nonspecific fluid phase endocytosis, specific LDL receptor-mediated endocytosis, or both mechanisms. In certain experimental models the endothelium appears to take up LDL almost entirely via a pathway independent of high affinity LDL receptors.[220]

The intense traffic present in each endothelial cell seems to have its own regulation, including the recycling of vesicles, the synthesis of new vesicles, the movement of vesicles to and from the plasma membrane, and the fusion of vesicles with the plasma membrane. A critical role in this regulation of membrane traffic is attributed to calcium, particularly as concerns the movement of vesicles to the subluminal surface and their fusion with the plasma membrane. In certain works, calcium levels appeared to play a crucial role in the regulation of both fluid phase and receptor-mediated endocytosis.

Lipoprotein macromolecules taken up by the nonspecific pathway may leave the endothelial cell more radily than those taken up by specific receptors. It might be presumed that metabolic changes occur when a specific macromolecule is taken up by specific pathways that do not occur or occur more slowly when it is taken up by nonspecific mechanisms. The receptor appears as a complex protein whose cytoplasmic component may be specialized to target it for fusion with the lysosomes; the nonspecific vesicles might have no such target signal, as their internalization does not require attachment of the microtubular system to the cytoplasmic portion of the receptor protein to facilitate the internalization of specific vesicles.

C. Atherogenic and Antiatherogenic Lipoproteins

1. General Observations

Under normal conditions, the lipoprotein pathway regulates the uptake, storage, synthesis, and removal of cholesterol and protects arterial intima from an overaccumulation which appears only when this regulatory mechanism becomes deficient or inadequate.

A delivery to the artery of LDL-cholesterol of about 2 to 9 μg/g wet tissue weight per day or 0.1 μg cholesterol per square centimeter of arterial surface per day was suggested.[257] If net delivery of cholesterol would continue at such a rate without concomitant removal of cholesterol by some mechanisms, its level in the arterial intima would build up to enormous values in a relatively short time. An adequate mechanism for reverse cholesterol transport exists in the arterial wall, as is the case with other tissues and organs. Under in vivo experimental conditions approximately 5 μg LDL was degraded daily per gram aorta net weight and if we assume that the entire LDL macromolecule was taken up as a unit, the rate of LDL-cholesterol delivery would be about 10 μg of sterol per gram daily.[196]

When the capacity of the cells to release stored lipids is exceeded by their capacity to take up lipoproteins, then local conditions exist for the occurrence of lipid-laden cells.

Analytical investigations suggest that two processes related to intracellular LDL penetration must be taken into consideration:

1. *The lipoprotein macromolecule binds to specific receptors* on the plasma membrane, followed by internalization by endocytosis and delivery of the LDL macromolecule to cell lysosomes
2. *Transfer of free cholesterol to cells independent of the net uptake of lipoproteins,* involving surface transfer of free cholesterol from the lipoproteins to the plasma membrane

Whereas the first pathway might be controlled by the cholesterol cellular content, which, by a negative feedback mechanism regulates the number of receptor able to bind LDL macromolecules and to limit the further uptake of cholesterol by cells, the second pathway is without adequate control and therefore more atherogenic. This refers to the arterial smooth muscle cells; on the other hand, in hepatic cells the catabolism of lipoproteins is stimulated following the activity of cell receptors.

In normal subjects, an important part of the circulating pool of LDL is cleared each day by the above-mentioned pathways. The major cause of a decrease in clearance of LDL seems to be a reduction in the activity of LDL receptors. Another factor affecting blood level of LDL is its production rate, since LDL is derived from the degradation of VLDL. When the activity of LDL receptors is high, less VLDL is converted to LDL because more is removed directly from the circulation. Both apparently distinct conditions, *a decrease in LDL receptors which leads to hypercholesterolemia and an overproduction of VLDL which is followed by an increased input of LDL,* may predispose to lipid accumulation within the arterial wall. An overproduction of VLDL followed by an augmented occurrence of LDL particles may not be associated with hypercholesterolemia, but the composition of LDL is abnormal.[258]

Several studies reported in recent years have shown that the lowering of serum cholesterol levels of diet and/or drugs will decrease the risk of myocardial clinical manifestations induced by coronary atherosclerosis. The Coronary Primary Prevention Trial[213,214] has provided the most convincing evidence in this respect. The results suggest that attempts must be made to lower LDL by 50% or more in hypercholesterolemic patients, and this requires stimulation of the activity of the hepatic receptors that bind this macromolecule and remove it from the circulation. The Coronary Primary Prevention Trial used cholestyramine which seems to augment the activity of hepatic LDL receptors. These data might indicate that *in the same individual and period of time coronary receptors for LDL may act as atherogenic agents*

and hepatic receptors for LDL as antiatherogenic agents. Drugs such as verapamil enhance receptor-mediated endocytosis of LDL, apparently due to an increase in receptor number. This effect may be related to the beneficial action of calcium channel blockers in experimental atherosclerosis by promoting transfer of LDL-cholesteryl esters from the interstitium to a catabolic compartment.[259]

A new line of investigation concerning the atherogenic role of lipids is related to the effect of lipoproteins and particularly of LDL on smooth muscle cell replication. Hyperlipemia, even of short duration, has been shown to induce in certain experimental models proliferation of arterial smooth muscle cells in intact animals. This excess cell proliferation seems to be reversible when plasma lipid concentration is lowered to initial levels by dietary means. Of note are also the observations on the proliferation induced by hyperlipidemic serum in stationary outgrowths from arterial explants, whereas the normal serum does not produce similar effects. This proliferations appears in the absence of platelets and of PDGF, suggesting that lipoproteins are directly involved in the proliferative components of atherosclerosis.[260,261]

An intriguing question is are there LDL subspecies of different structure and composition which might also have different metabolic and atherogenic roles? In this connection it is tempting to assume that certain human LDLs bind preferentially to the intimal ground substance and fibers — others exert proliferative effects or act only as a source of cholesterol. In some experimental models high molecular weight hypercholesterolemic LDL appeared to be two to three times more atherogenic than was the same amount of normal LDL.[262] The enhanced atherogenicity of these large LDL particles was related to the ability of hypercholesterolemic LDL to produce cholesterol accumulation in arterial smooth muscle cells, being considered about twice as effective as normal LDL in stimulating both cholesterol accumulation and esterification.

Certain investigators believe that cholesterol itself is not an important atherogenic agent, but some oxygenated lipids, such as 2,6-hydroxycholesterol and oxidized fatty acids, are considered important.[220] Products of lipid peroxidation have also been reported to inhibit the PGI_2 pathway in the arterial wall and to favor the accumulation of lipids in lesions, this accumulation contributing to the formation of lipid peroxides. A positive correlation was detected between the apparently increased levels of lipid peroxides and atherogenesis.[263] On the other hand, there are studies which demonstrate that hypercholesterolemia does not stimulate the onset of fibrous plaques and that the important amount of collagen present in atherosclerotic lesions may be partly the result of an inherently greater potential of smooth muscle cells to synthesize collagen.[264]

An additional atherogenic role of lipoproteins could be due to their capacity to alter arterial wall responsiveness to a number of vasoactive agents, particularly an augmented pressor response to norepinephrine, serotonin, ergonovine, histamine, etc. This effect was related to changes in the activity of Na^+-K^+ ATPase or other membrane enzyme systems able to increase the contractility of arterial smooth muscle cells.[265] A significant contraction may lead to spasm occurrence, altered blood flow, impairment of myocardial blood supply, and may also favor thrombus formation.

2. Lipoprotein Interactions

Lipid transport is solved by the esterification of sterol with long chian fatty acids and packing these esters within the hydrophobic cores of plasma lipoproteins. With its polar hydroxyl group esterified cholesterol remains sequestered within this core, whereas small amounts of unesterified cholesterol on the surface of the particle are maintained in an equilibrium exchange with the cholesterol of cell membranes. As concerns the larger amounts of cholesterol esters sequestered within the core of the particle they can only leave the respective particle by means of highly controlled processes.

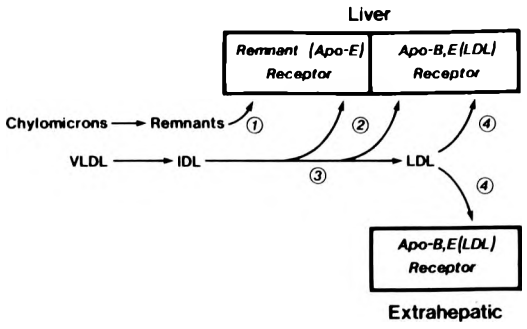
The human major lipoproteins were considered several decades ago to be independent particles, each with its own pathway of synthesis, metabolism, and degradation. Recent research findings are altering this concept, all plasma lipoproteins occurring as interrelated parts of one or more metabolic cycle which together are involved in plasma lipid transport. A dynamic equilibrium seems to exist among these interrelated particles, chylomicrons and VLDL being the primary secretory products of cells. Chylomicrons are composed primarily of triglycerides and originate in the intestine from exogenous (dietary) fat, being rapidly metabolized in the bloodstream. VLDL is the main carrier of endogenous triglycerides, originates in the liver and small intestine, and is rapidly metabolized to a transient intermediate form which is further degraded into LDL in the bloodstream. These LDL particles are mainly involved in atherogenesis.

Triglyceride-rich chylomicrons containing apo B-48 are secreted by the intestine, rapidly aquire apo E and apo C-II from HDL, and undergo hydrolysis by lipoprotein lipase. Following hydrolysis, chylomicron remnants are ultimately taken up by the liver via an apo E-mediated receptor system. Lipoprotein lipase hydrolysis leads to a progressive increase in cholesteryl esters as a major core lipid; cholesteryl esters are also formed via the action of lecithin-cholesterol acyltransferase (LCAT) in HDL.

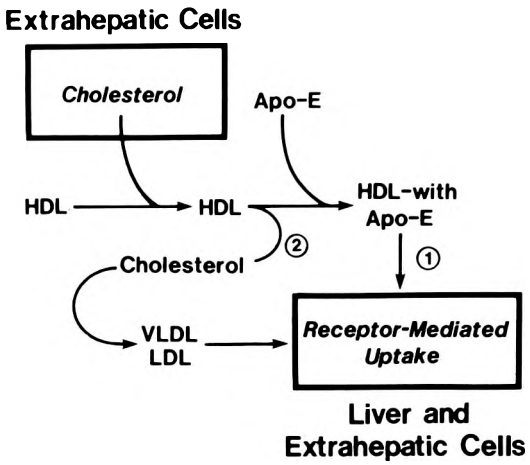
A second important step involves apo B-100 containing triglyceride-rich VLDLs, which also acquire apo E and apo C-II from HDL and undergo hydrolysis, giving rise to LDL and remnants. These last products are taken up by the liver via apo E and LDL receptors. Apo B-100 containing LDL interact with high affinity LDL receptors both in the liver and in peripheral cells, initiating receptor-mediated endocytosis and LDL catabolism.

A third important step refers to the formation of HDL particles, both the liver and the intestine being involved in their production. HDL is initially formed as a discoidal-shaped precursor lipoprotein, composed of phospholipids, apoproteins, and small amounts of free cholesterol, and is converted to the mature spherical particle after interaction with LCAT. The second potential source of HDL precursor is considered the surface coat of lipolyzed triglyceride-rich lipoproteins (transfer of VLDL and chylomicron constituents to HDL during the course of lipolysis). Certain investigators present HDL as a modified product of rebundant surface lipids and proteins generated during the process of triglyceride transport, namely during chylomicron and VLDL lipolysis.[266] HDL particles range in diameter between 70 and 100 Å and in molecular mass between 200 and 400 $\times$ 10^3 daltons. Discrete HDL populations are continuously detected and may represent features of thermodynamically favorable structures. The contribution of cholesterol to the total mass is only 15% and according to certain views, it is difficult to define HDL as a vechicle for lipid transport; rather it could be defined as the site of plasma cholesterol esterification. A discoidal-shaped precursor first appears, including HDL aproprotein, lecithin, and free cholesterol, which is transformed into spherical particles involving the action of LCAT, which results in the formation of cholesteryl esters from free cholesterol. The cholesteryl esters then enter the core of the particle. Two decades ago a view was advanced in which HDL transports cholesteryl esters from the coronary wall to the liver.[267] Additional free cholesterol could then be received by HDL from myogenic and macrophage foam cells as an important step of this reverse cholesterol transport. Some evidence is accumulating that HDL may reduce the arterial lipid, not only by cholesterol transport, but also by interferring with LDL binding to the cell surface.[268] In addition, as already mentioned, HDL has the ability to act as a scavenger during the intravascular lipolysis of chylomicrons and VLDL. Availability of HDL may determine the amount and kind of debris deposited in the reticulo-endothelial cells, including those present in atherosclerotic lesions.

A general scheme of lipoprotein metabolism and of the role of HDL in acquiring cholesterol from cell containing excess cholesterol is presented in Figure 59.[193] Likewise, an attempt to present VLDL-LDL pathways and reverse cholesterol transport in diagrams is illustrated in Figure 60.[269]



A

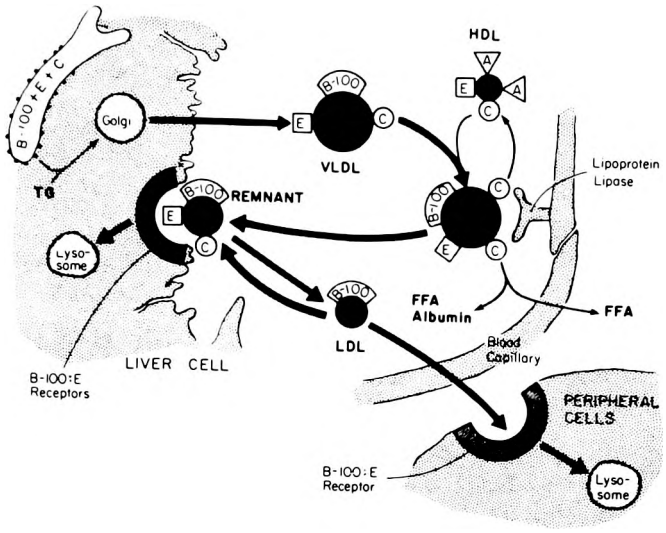


B

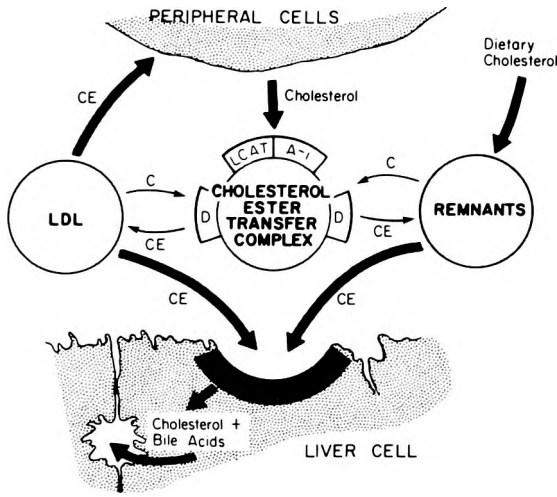
FIGURE 59. (A) General scheme of lipoprotein metabolism. (B) Role of HDL in acquiring cholesterol from cells containing excess cholesterol and redistributing the cholesterol to cells requiring it. (From Mahley, R. W., *Circulation*, 72, 943, 1985. With permission.)

Strongly related to these complex stages of lipoprotein metabolism are the specific actions exerted by the apoproteins, a group of water-soluble polypeptides able to influence lipid synthesis, transport, and degradation. They are included in the outer shell of the lipoprotein particle together with phospholipids and free cholesterol. The concentration of apoproteins and surface lipids is relatively constant, but the type of the prevalent apoprotein is different from one lipoprotein particle to the other: A-I, A-II, A-III, A-IV, B, C-I, C-II, C-III, D, E, F, etc.

Many human plasma apoproteins have been isolated and characterized; the sequence of amino acids of the majority of these compounds and specific functions are determined. They can function as cofactors for enzymes involved in lipoprotein metabolism (apo C-II, for instance, is a cofactor for lipoprotein lipase, apo A-I a cofactor for acetylcholesterol acyl-



A



B

FIGURE 60. (A) Pathways of chylomicron-VLDL and LDL metabolism and (B) reverse cholesterol transport. These are major pathways by which cholesterol in the surface coat of plasma lipoproteins and in cells is transported to the liver for excretion in the bile. (From Havel, R. J., *Med. Clin. North Am.*, 66, 319, 1982. With permission.)

transferase or ACAT); they also act as ligands on the lipoprotein particle for interaction with a specific cellular receptor (apo B-100 in the LDL pathway, apo E in the chylomicron and chylomicron remnant pathway) and as a structural protein for synthesis of the lipoprotein macromolecule (apo B-100 for VLDL in the liver, apo B-48 for chylomicrons in the intestine, and apo A-I for HDL).

Of particular interest for the suggested atherogenic role of lipids is the mediated interaction with the specific receptor and the capacity to activate the lipoprotein lipase system and lecithinocholesterol acyltransferase. Likewise, of particular interest for atherogenesis is the presence of apo A-I located on the surface of HDL and which plays a major role in binding free cholesterol. Apo A-I, together with the cholesteryl ester exchange protein, transfers cholesterol esters from the core of the HDL particle to other particles. The continuous formation of free cholesterol in intimal cells leads to the existence of an important amount of free cholesterol which is available for transport out of cells. This free cholesterol has the ability to migrate through the cell membrane which becomes quickly saturated with cholesterol and apo A-I acts as an acceptor material on the outside of the membrane. This process seems to be continuous, its rate directly dependent on the number of available apo A-I binding sites. Many results suggest that apo A-I is not only an activator of lecithincholesterol acyltransferase, but plays a major role in regulating the rate of removal of cholesteryl esters from atherosclerotic lesions. Some investigators think that the level of apo A-I might be considered to be a better marker for atherosclerotic involvement than the level of HDL-cholesterol, because HDL-cholesterol measurements include HDL particles that are fully saturated with cholesterol.[216] A correlation was detected between the level of apo A-I and the severity of atherosclerotic involvement in patients undergoing coronary angiography.[270] Genetic analysis of apo A-I levels in patients with a strong positive family history of coronary heart disease has shown that 90% of the variability of apo A-I can be explained on the basis of a single gene locus. This would indicate that apo A-I is an important marker of the "genetic risk for the development of atherosclerosis".[216] Also of note is the observation that a gradual decrease in the HDL proportion among other lipoprotein particles can be detected in the course of animal evolution and that this diminution is associated with an augmented susceptibility to atherosclerotic involvement.[271]

Likewise, apo B and LDL-cholesterol levels show a strong relationship to the extent and severity of coronary atherosclerosis, but not with the degree of luminal narrowing.[272] According to other views and results "it is perhaps naive to expect that the measurement of the plasma concentrations of either plasma lipids or apoproteins would be strongly related to the severity of atherosclerotic lesions".[273] Our own experience supports this view and we are amazed to read in the available literature that the concentration of apoproteins may differentiate among middle-aged subjects cases with from cases without atherosclerosis. Such cases without atherosclerotic involvement among middle-aged subjects of industrialized countries must be presented as curiosities.

The role in atherogenesis of the C-III apoprotein which serves as a regulator of the activity of lipoprotein lipase system and as an inhibitor of the interaction of triglyceride-rich lipoproteins with liver receptors is not clearly delineated. As concerns apo D, it seems to be similar with the plasma cholesterol ester transfer protein.

To summarize, *each lipoprotein particle is characterized by a specific apoprotein profile which determines particular metabolic events during the life span of the respective lipoprotein in plasma and in tissues.* Apoproteins are involved in lipoprotein secretion, which leads to the occurrence of nascent lipoproteins. They then are involved in the penetration of lipoproteins in the circulation, followed by the intravascular change of lipoprotein components (during this monenzymatic change certain apoproteins such as apo E and apo A-I will be transferred from VLDL to HDL, while others such as C, will be transferred from HDL to VLDL). In an additional, enzymatic step, LDL particles are formed from VLDL as well as VLDL remnants and surface components which are released and transferred to HDL.[274]

This highly dynamic system of lipid and protein exchange among various types of lipoproteins is strongly influenced by genetic disorders. Heritable disorders in apoprotein structure, pattern of synthesis, metabolism and degradation, and in their affinity for receptors are partly defined at the genomic level. Thus, for instance, the basis for familial hypercho-

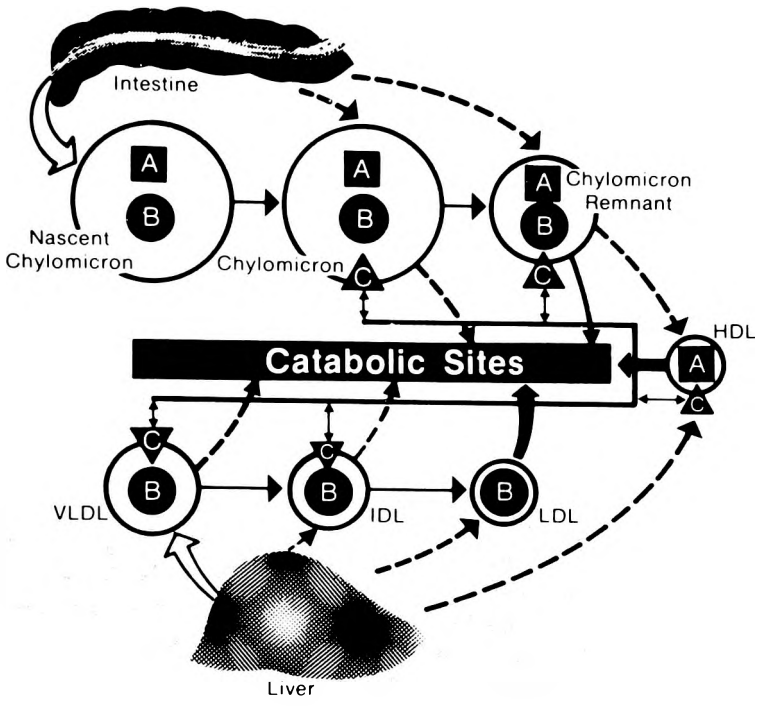


FIGURE 61. Conceptual view of A, B, and C apoprotein metabolism. (From Levy, R. I. and Rifkind, B. M., *Circulation*, 62(Suppl. 4), 1, 1980. With permission.)

lesterolemia was suggested to be a single abnormal allele of the genes for the LDL-apo B receptor. Genetic defects were also revealed in families with members that have plasma apo A-I and apo C-III deficiency. These patients show a rapid and severe development of coronary atherosclerosis with a large spectrum of clinical manifestations, in spite of an apparently normal LDL level. Similarly, subjects with normal plasma LDL levels might have a genetic defect in an HDL-mediated cholesterol transport pathway. It was repeatedly demonstrated that the cholesterol-carrying protein apo A-I, in the presence of phospholipid and lecithin cholesterol acyltransferase acts to form HDL which transports cholesterol from the artery wall back to the liver. Lecithin cholesterol acyltransferase interacts with HDL, increases the cholesterol ester content in this lipoprotein, and depletes the free cholesterol concentration at the HDL surface. This is followed by the occurrence of an adequate gradient for free cholesterol between the membrane of arterial smooth muscle cells or macrophages and the LDL surface. Free cholesterol will move up from the plasma membrane to HDL and form a free-cholesterol enriched particle. Lecithin:cholesterol acyltransferase will react with this enriched particle and a complex process will take place, cholesteryl esters will be transferred to VLDL, and will reach the liver as VLDL remnants. The above data may explain why defects in the gene for apo A-I could be important in intraarterial lipid accumulation.

A conceptual view of A, B and C apoprotein metabolism is presented in Figure 61.[275]

In spite of important progress in our knowledge, there is scant information on the way cholesterol escapes from the cell and the involvement of this mechanism in atherogenesis. HDL seems to be the unique lipoprotein able to produce reverse cholesterol transport, but its real role in the natural history of human coronary atherosclerosis remains a subject of speculation.

On the other hand, there is large agreement that LDL transports 60 to 75% of the plasma cholesterol to the cells and is the main lipoprotein macromolecule involved in atherogenesis. The delivery of cholesterol and particularly of cholesterol esters which are too hydrophobic

to pass through membranes is based on the existence of specific receptors strategically located on the intimal smooth muscle cell surface. These receptors include a glycoprotein able to bind two proteins: apo B-100, the main protein of LDL, and apo E, found in multiple copes in IDL and an HDL subclass. An endocytosis cycle seems to occur at the site of each LDL receptor continuously, whether it is occupied by LDL particles. The LDL that dissociates from the receptor is delivered to a lysosome when the membranes of the endosome and lysosome fuse. The next step is the hydrolysis of amino acids by proteases, whereas cholesterol esters are hydrolyzed by an acid lipase, liberating cholesterol. This pathway requires the continuous movement of a membrane-embedded protein from one organelle to another in a highly ordered fashion. There are particular cases in which a slow transport from endoplasmic reticulum to Golgi apparatus occurs, or in which receptors are processed and reach the cell surface but fail to bind LDL normally, or bind LD but fail to cluster in coated pits.

The receptor binds LDL optimally when the lipoproteins are present at a cholesterol concentration of 25 mg/100 mℓ; this would indicate that a level of LDL-cholesterol in blood plasma of only 25 mg/100 mℓ would be sufficient to deliver cholesterol to all cells and it corresponds to a total serum cholesterol level from 110 to 150 mg/100 mℓ. Only the LDL levels in neonates and infants can be considered to be appropriate for physiologic receptor binding. When these levels augment, the receptors become saturated with LDL particles and this saturation represents the upper limit of the rate at which LDL can be removed efficiently from plasma. Since each specific receptor can handle only one LDL macromolecule at a time, this saturation incites an additional clearance carried out by nonreceptor pathways; *these alternate pathways are strongly atherogenic because they are unable to limit the removal of LDL from plasma,* as is the number of specific receptors.

Two properties of the receptor, its high affinity of LDL and its ability to cycle multiple times in and out of the cell, allow large amounts of cholesterol to be delivered to smooth muscle cells of arterial intima.

The cholesterol generated from LDL within the lysosomes:

1. Suppresses HMG-CoA reductase activity
2. Activates a cholesterol esterifying enzyme (ACAT) so that excess cholesterol can be stored in the cytoplasm
3. Suppresses the synthesis of LDL receptors by lowering the concentration of receptors for messenger RNA
4. Allows the cell to adjust the number of LDL receptors to provide sufficient cholesterol for metabolic needs, without causing overaccumulation

As long as the smooth muscle cells keep their level of unesterified cholesterol remarkably constant, lipid accumulation does not occur, receptors moving from one organelle to another in a coherent biochemical pathway which links the cell surface to the endosome and the lysosome.

The therapeutic implications of the LDL receptor studies center on strategies for increasing the production of LDL receptors in the liver, thereby lowering plasma LDL levels.[276] The plasma LDL level decreases as a result of the increase in hepatic LDL receptors; conversely, whenever the number of receptors is reduced, plasma LDL levels must rise. Consumption of a high-fat diet decreases the number of LDL receptors in the liver and contributes in this way (saturation and suppression) to the onset of high cholesterol levels in the circulating blood. If lysosomal function is altered, the liberation of free cholesterol is compromised, undegraded LDLs accumulate within the cell, and the structural organization of membranes is drastically changed, as discussed in the previous pages. At the opposite end is an excessive degradation of cholesterol esters followed by the presence of abnormal amounts of free cholesterol which are incorporated in the membranes.

A mechanism called retroendocytosis was also suggested to play a role relating cholesterol and atherogenesis. This term refers to the reexcretion from the cell of the LDL particles not degraded in the lysosomes.[277] The retroendocytosis pathway may provide a mechanism for altering cholesterol metabolism of arterial smooth muscle cells without requiring the degradation of the entire LDL macromolecule.

In essence, the uptake of LDL particles by way of high affinity receptors of the intimal smooth muscle cells seems to play a certain role in atherogenesis when this uptake is altered, but its significance as concerns human coronary atherosclerosis is not clear. Patients with homozygous familial hypercholesterolemia of the receptor negative type develop striking premature coronary atherosclerosis and often die in the first or second decades of life from coronary heart disease. In such unfortunate cases, receptor deficiency is responsible for the increased plasma concentration of LDL, but specific uptake via the LDL receptor is not necessary for an accelerated onset and progression of atherosclerotic lesions. These patients also have abnormally high levels of IDL, resembling VLDL remnants and their possible role in atherogenesis was suggested. This view is supported by studies carried out on patients with type III hyperlipoproteinemia; they have both hypertriglyceridemia and hypercholesterolemia and their plasma is very rich in chylomicrons and VLDL or IDL, but poor in LDL and HDL. The underlying genetic defect responsible for this hyperlipoproteinemia is an abnormal form of apo E. It does not bind normally to either the apo B, E, (LDL), or remnant (apo E) lipoprotein receptors. The mechanisms responsible for accelerated atherogenesis and progression of atherosclerotic lesions in both coronary and peripheral arteries in subjects with type III hyperlipoproteinemia is not clear. Peculiar features are the low levels of LDL and HDL and the intense participation of macrophages as scavenger cells. Both chylomicrons and VLDL remnants bind to macrophage receptors and cause massive cholesterol ester accumulation.[193]

The role of other types of hyperlipoproteinemia and lipid metaoblism abnormalities in atherogenesis and progression of atherosclerotic lesions remains a subject for speculation. The decrease in cholesterol acceptor and transport functions of HDL is, for instance, considered a possible atherogenic mechanism, since it may lead to excessive cholesterol accumulation; this decrease was mainly related to alterations in the molecular organization of the HDL surface monolayer.[278] Such alterations may lead to a diminution of the so-called antiatherogenic function of HDL, namely a decreased ability to accept cholesterol from the membrane of intimal cells, from platelets, and from VLDL; additionally, there is a reduced activity of LCAT reaction, whereby free cholesterol is transformed into esters.[279] The complexity of the actions exercised by HDL and their relationship to atherogenesis appears more clear if these actions are analyzed at the level of the individual cell, arterial wall, and blood plasma.[280] The interest in this lipoprotein and in raising its level by different means stem from the well-established low incidence of ischemic events in subjects with elevated HDL-cholesterol. Attempts to produce drug agents to this end have been numerous in the past few years.[281,282]

It might be of interest to recall that VLDL, LDL, and HDL transport not only cholesterol, but also spontaneously occurring oxidation derivatives of cholesterol, having a biological effect quite different from that of purified lipid. These spontaneously occurring oxidation derivatives of cholesterol have been found in cholesterol containing food, in powdered eggs, meat, etc. In vitro they show an unexpected toxicity to a variety of cell cultures while purified cholesterol does not produce similar effects.

D. Significance of Lipid-Laden Cells

1. Lipid-Laden Cells of Smooth Muscle Origin

Evidence accumulating during the past decades suggested that smooth muscle cells are the principal arterial wall component trapping and processing cholesterol-rich lipoproteins

which enter from the arterial lumen and that smooth muscle cells also produce the extracellular matrix and the intimal connective tissue. More than 2 decades ago, two types of cell populations were isolated: (1) atherophil cells, with considerable activity of respiratory enzymes of the Krebs cycle, capable of rapid in vitro incorporation of lipids and also of rapid conversion into lipid-filled cells and (2) fibrophil cells, with a very limited capacity to incorporate lipids, but able to give rise to matrix components.[283]

In other studies, five types of smooth muscle cells were described: mature, intermediate, fibroblast-like, primitive, and large cells containing lipid droplets.[284] Emphasis was also placed on the capacity of these cells to migrate and proliferate and to behave as multifunctional mesenchymal cells.[115,285] Many other data on the physiology and pathophysiology of arterial smooth muscle cells, as related to the natural history of coronary atherosclerosis, are presented in Section III.A—D.

A doubly ligated artery injected with Ringer's solution into the lumen showed, in animals kept on a normal diet, progressive accumulation of lipid droplets in smooth muscle cells. These droplets were present in 25% of these cells 1 day after ligation, then decreased thereafter. When doubly ligated artery was filled with either human LDL or VLDL, the percentage of cells with intracellular lipid droplets increased to a maximum 85% 4 days postligation.[286,287] Intracellular accumulation of cholesterol was greater following the injection of LDL than VLDL. Electron micrographs of lipid-filled cells occurring in the presence of LDL particles revealed vacuoles with halos of electron-dense limiting membrane and lamellated bodies.

Studies dealing specifically with the organization of these myelin figures showed that cholesterol and other nonplar lipids are dispersed within the hydrophobic parts of the bimolecular leaflets of phospholipids. The dark bands of the lamellated structure seen on the electron micrographs of lipid-filled arterial smooth muscle cells would represent the aqueous phase with the ionizable groups of phospholipids and proteins embedded in it. The light bands of the lamellated structures would include cholesterol and cholesteryl esters dispersed within the long hydrocarbon chains of the phospholipids.[288] In this hypothetical model, the aqueous phase is the site of enzyme activities, leading to cholesterol esterification or removal. In an early stage of lipid-filled smooth muscle cell formation, the micellular structure is more or less similar to that of pure lecithin-cholesterol mixtures. In a subsequent stage of evolution, the respective structures became partly lamellated and partly amorphous, and these were considered as intermediate forms between the liquid-crystalline droplets and the truly cholesteryl ester globules. In an even more advanced stage of lipid-laden cell formation, amorphous cholesteryl ester globules predominate, whereas very few lamellated liquid-crystalline droplets remain.[289] These accumulations in smooth muscle cells of cholesteryl ester globules occur as spherical inclusions with a structural organization of a lyotropic smectic mesophase (liquid-crystal) secondarily organized into multiple concentric lamellae.[290] They are designated in the available literature as fat droplets, double refractile droplets, myelin figures, myelin-like bodies, anisotropic spherocrystals, fluid spherocrystals, paracrystalline spherulites, anisotropic globules, globular fat, birefringent droplets, etc. All these terms refer to structures with some level of crystallinity and radial symmetry, the prevalent organization being that of a liquid crystal mesophase.

Large (0.4 to 0.6 μm) cholesteryl ester-rich lipid droplets have been isolated from lipid-laden cells of arterial intima, including 95% cholesteryl ester and oleate as the predominant acyl component.[291] Smaller (0.24 to 0.36 μm) cholesteryl ester-rich particles were also isolated, comprising 22 to 39% cholesteryl ester as lipid and linoleate as the major cholesteryl ester acyl component.[292,293] The larger cholesteryl ester droplets occurred mainly within smooth muscle cells, the smaller prevailed in the intercellular space. The use of filipin demonstrated that unesterified cholesterol was mainly present in intercellular spaces, being accumulated in the subendothelial area and its occurrence seemed to precede morphologic

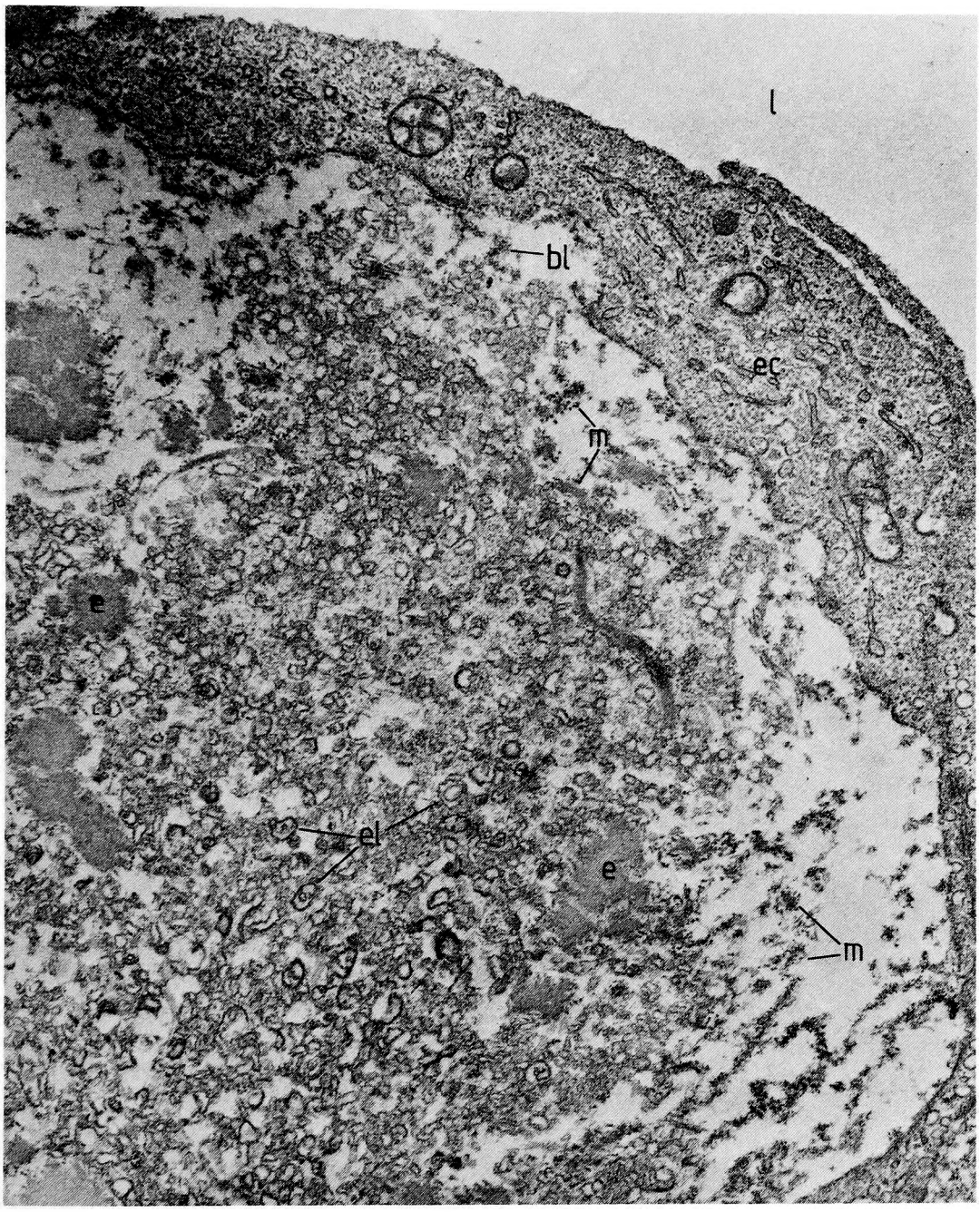


FIGURE 62. Aortic arch from a hyperlipidemic rabbit fed a cholesterol-rich diet for 2 weeks. In a morphologically lesion-free area, the intima contains numerous extracellular phospholipid liposome-like structures (el) trapped in a proliferated matrix predominantly made of basal lamina-like material (bl) and bundles of elastin (e). (ee) Endothelium; (l) lumen; (m) microfibrils. (Magnification × 19,800. Photo courtesy of Nicolae Simionescu).

changes in the endothlium and the first clusters of lipid-laden cells.[294] This accumulation of subendothelial unesterified cholesterol which did not produce visible endothelial injury was associated with migration of monocytes into the subendothelial space. Similar accumulations of unesterified cholesterol were revealed in tendon xanthomas.[294,295] The continuous occurrence and deposition of small (100 to 300 μm) vesicles formed by phospholipid lamellae rich in unesterified cholesterol were revealed in the subendothelial region of aorta of cholesterol-fed rabbits; these structures were tentatively called extracellular liposomes (Figures 62 to 64).[241]

FIGURE 63. Specimen similar to that in Figure 62, but prepared by the freeze-fracture technique. The extracellular liposomes (el) display smooth-surface surfaces devoid of translamellar particles. (Magnification × 68,250. Photo courtesy of Nicolae Simionescu).

The above results would indicate that one of the first changes during the occurrence of lipid-filled cells of smooth muscle origin is a stimulation of cholesterol esterification. Other suggested changes would be an increase in the negative charge within the ground substance, binding of LDL by electrostatic forces (phospholipids via calcium), a change in the charge distribution of the LDL macomolecule, and transition of the liquid core of LDL into a liquid crystalline organization.[295]

In certain experimental models, one of the first and main changes (secondary to the uptake

FIGURE 64. Specimen similar to that in Figure 63, but incubated with filipin (known to bind specifically to 3-β-hydroxysterols). Extracellular liposomes are marked by characteristic 20 to 25 μm protrusions representing filipin-sterol complexes (arrows). (em) Extracellular matrix. (Magnification × 58,240. Photo courtesy of Nicolae Simionescu).

of LDL) is the above-mentioned cholesterol esterification stimulation; it appears to be associated with an intensification of phospholipid and trigyceride synthesis.[297] It is tempting to presume that in a cell which will become lipid-laden, an excess of LDL particles binds to receptors; the complexes' receptor-LDL particles then invaginate to form endocytic vesicles which migrate through the cytoplasm until they fuse with a lysosome where LDLs are exposed to a variety of hydrolytic enzymes. In such elements destined to become lipid-laden cells, the resulting free cholesterol does not turn off the synthesis of the LDL receptor itself and this is not followed by a diminution of LDL entry into the smooth muscle cells preventing or delaying overaccumulation of lipid droplets. On the other hand, the free cholesterol activates a cholesterol-esterifying enzyme of smooth muscle cells, ACAT or acetyl coenzyme A, and cholesterol acetyltransferase able to reesterify free cholesterol and to store it as intracellular droplets. According to the above-mentioned view, in order to utilize the cho-

lesterol contained within the LDL core, smooth muscle cells must be able to take apart the LDL particle and hydrolyze the cholesteryl esters so as to generate free cholesterol. Likewise, a possibility exists for the cell to take free cholesterol directly from the surface of lipoprotein particles or from extracellular liposomes.[241] A model system was proposed in which enrichment of smooth muscle cells with cholesteryl esters can occur, bypassing receptor-mediated uptake of LDL or apo E-rich lipoproteins. Such a mechanism should not be susceptible to the autoregulation of an apo B-E receptor-mediated process and its existence could explain one of the pathways leading to the conversion of a smooth muscle cell into a lipid-laden cell.[298] Other mechanisms seem also to exist, the available evidence indicating that arterial smooth muscle cells have an apo B receptor system based on a feedback mechanism, take up relatively large quantities of LDL and store some of these particles in specific vacuoles, and use an unregulated nonreceptor scavenger mechanism to take up lipids.[299-302]

Plasma LCAT is the enzyme responsible for the formation of virtually all of the cholesteryl esters in human plasma in the postabsorptive state. As a result of this enzymatic activity upon nascent HDL particles, a gradient appears favoring the movement of cholesterol from arterial smooth muscle cells filled with lipid droplets into the blood plasma. Nascent HDL in the form of bilayer discs has been shown to accept cholesterol from the lipid-filled cells of arterial intima, whereas LCAT deficiency states of genetic origin or secondary to liver disease favor the accumulation of LDL particles in intimal smooth muscle cells.

In certain experimental studies, intracellular lipid accumulation is considered to result, at least in part, in altered cholesteryl ester metabolism in smooth muscle cells.[303] This metabolism seems to be under the influence of endothelial cells which would modulate cholesterol accumulation. The relative increase in cholesteryl esters in re-endothelialized areas of injured arteries may result from variations in enzymatic activities concerning cholesterol esterification and cholesteryl ester degradation in injured and uninjured areas. Diets can also influence the occurrence of lipid-laden cells of smooth muscle origin; if they are rich in saturated fat and cholesterol they could stimulate the production of chylomicrons and VLDL remnants and could lead to impaired catabolism of LDL by suppression of apo B receptor. There is also some evidence that monocytes and neutrophils may oxidize LDL particles which acquire a more important cytotoxic character.[304] Likewise, retroendocytosis was detected, in which LDL particles or their components can be reexcreted from smooth muscle cells filled with lipid droplets.[277] This mechanism of retroendocytosis or reverse endocytosis seems to be mainly used by cells in the presence of LDL particles exhibiting a cytotoxic character to prevent the possible occurrence of necrosis as a result of the accumulation of large amounts of cytotoxic lipids. As previously mentioned, studies have drawn attention to the effects of oxidized forms of cholesterol, peroxides of polyunsaturated fatty acids, and a number of avenues by which free radicals of oxygen might be liberated into a developing lipid-filled smooth muscle cell.

2. Lipid-Laden Cells of Monocyte-Macrophage Origin

There is continued investigation and progress concerning the monocyte-macrophage as a contributor to atherogenesis. Current observations indicate that few monocyte-derived macrophages are present in human atherosclerotic lesions, unless there is damage to the artery wall by antigen-antibody complexes or other immunologic mechanisms, specific diseases of the reticulo-endothelial system, and other particular conditions. Histochemical tests suggest that fewer than 10% of cells present in human arteroscleroticlesions are of monocyte-macrophage origin.[305] On the other hand, observations in the hypercholesterolemic monkeys, pigs, rabbits, and other laboratory animals show that macrophages contribute to the development of early lesions by virtue of their normal function as scavengers.[306] A frequently mentioned view dealing with experimental atherosclerosis is that monocyte-macrophage cells

may take up lipoprotein particles either in the bloodstream, or in the spleen, liver, lymph nodes, bone marrow, skin, myocardium, etc., and that they then accumulate in the subendothelial region of large arteries giving rise to fatty streak-like lesions.[307-313] Particularly in cholesterol-fed rabbits, the lipid-laden cells have been regarded as reticulo-endothelial elements which have migrated via the bloodstream from the aforementioned organs to the arterial wall. Evidence of such migration was founded on: the presence of radioactive labeled macrophages in early atherosclerotic lesions, the results of studies using India ink injected intravenously, the results of investigations which employed various histochemical tests for blood macrophages, and the light and electron microscopic pictures of these lipid-laden cells suggesting the penetration of blood macrophages overloaded with lipid droplets through the arterial endothelium. An other important argument was furnished by the presence in aortic atherosclerotic lesions of the recipient rabbit of mononuclear cells of the thymidine-labeled donor.[314] Likewise, suggestive were the results of certain chemical investigations showing that peritoneal exudate cells from cholesterol-fed rabbits consisting of macrophages and small lymphocytes had an average cholesterol content four to eight times greater than macrophages obtained from control animals.[315]

In the hypercholesterolemic pig, atherosclerotic lesion development was preceded by intimal penetration of blood-borne mononuclear cells, medial smooth muscle cells not being apparently involved in the formation of early fatty-rich lesions.[316,317] In swine, in the early lipid-rich lesions that did not progress, the monocyte-macrophage appeared as the major source of lesion cells, whereas in advanced lesions the smooth muscle cell predominated. As long as lipid-laden cells of monocyte-macrophage origin prevailed and metabolized cholesteryl esters, the passage from early to advanced lesions was rarely seen.[317]

A mechanism worthy of consideration in atherogenesis is a monocyte clearance system able to send a large number of circulating monocytes to invade the intima in lesion-prone segments and to accumulate lipid droplets. Some of these lipid-laden macrophages migrate back into the bloodstream by crossing the arterial endothelium. The ratio of penetrating monocytes to emerging monocytes from early lipid-rich lesions decreases until a 1:1 ratio is achieved and the lesion progresses no further.[146] Such lesions in which cell immigration equals cell emigration appear stable and can be reactivated if a new period of hyperlipidemia occurs and the monocyte-macrophage clearance system becomes inadequate to remove intimal lipids. In such particular conditions, medial smooth muscle cells begin to migrate into the subendothelial area and to play an additional role as macrophages.

In many experimental models of atherosclerosis carried out to analyze the onset of early lesions, many of the lipid-laden cells are monocytes furnished by the flowing blood, or macrophages furnished by the Kupffer cells of the liver, alveolar and peritoneal macrophages, macrophages of the reticulo-endothelial system, microglial cells, and of other origin. These macrophages serve both as scavenger and secretory cells, as regulators of lymphocyte functions, and play a major role in inflammation. Some of these macrophages accumulated in intimal connective tissue of arteries contain catalase and lysosomal hydrolase levels comparable to those recorded in inflammatory areas.[318] These enzymatic activities are substantially greater than those found in smooth muscle cells. In vitro studies revealed that macrophages secrete collagenases, elastase, and various proteases and proteoglycan-degrading enzymes, which would indicate that macrophages can disintegrate the components of the intimal connective tissue. A wide variety of other potent biological products secreted by macrophages might be involved in atherogenesis, such as various prostacyclins, thromboxanes, leukotrienes, and many other products. An important observation is that the secretion of neutral proteases is augmented by phagocytosis of foreign particles, ingestion of immune complexes, lipid overloading, and prostaglandins. This would mean that lipid overload may be associated with the release of lysosomal hydrolases into the subendothelial region or thickened intimas; if the local pH falls, these enzymes could degrade collagen and elastic fibers, ground

substance, and other matrix components, and attack smooth muscle and endothelial cells. In addition, they could release macrophage-derived growth factors which seem to be involved in the proliferative phase of experimental atherogenesis. Activated monocyte releases more growth factors than the nonactivated, and this excretion may be influenced by local leukocytes accumulated at sites of arterial injury.[319]

Temporary adhesion of many blood monocytes to an area of arterial injury could, by local release of growth factors, actively participate in the development of an accelerated lesion. Another role of the blood monocytes in atherogenesis involves their immunologic properties and functions, and it is possible that the presence of monocytes and lymphocytes in the normal intima reflects the response to a chemical message.[320] The adhesion of monocytes to areas with endothelial injury seems to be mediated via the interaction between intimal IgG and the monocyte Fc receptor.[321] Arterial wall macrophages derived from blood monocytes also have receptors for both chylomicron and VLDL remnants and for chemically modified LDL.

The presence of receptors to modified LDL in macrophages,[322,323] has renewed the early interest in the role of the reticulo-endothelial system in the occurrence of lipid-laden cells having a different functional and pathophysiological significance than that of lipid-laden cells of smooth muscle origin.

In vitro, macrophages do not accumulate massive amounts of cholesteryl esters after incubation with high concentrations of LDL. On the other hand, these macrophages may become overloaded with cholesteryl esters in response to high LDL levels if chemically modified. This modified LDL can be recognized by unique receptors on macrophages; in particular, LDL modified by acetylation is avidly taken up by the acetyl LDL receptors. Such chemically modified LDLs could occur in plasma or in the arterial intima as they perfuse through the endothelium; there is some evidence that the action of monocytes themselves may produce some changes in LDL particles which acquire a more important cytotoxic character.[304] This type of lipoprotein modified by acetylation, acetoacetylation, or oxidation being easily recognized by the receptors of the macrophage may accumulate in the cytoplasm which progressively appears very rich in cholesteryl esters and in lipid droplets. Massive accumulation of cholesteryl esters can be stimulated in macrophages either by incubation with β migrating VLDL or with altered LDL. Alteration of the lysine residues of LDL by acetylation, acetoacetylation, treatment with malondialdehyde, and other compounds was followed by an enhanced uptake and accumulation of cholesteryl esters in macrophages.

Also of note is the finding that LDL complexes to certain components of the matrix of the thickened intima, particularly glycosaminoglycans and proteoglycans, can be more easily recognized and taken up by macrophages, giving rise to the occurrence of lipid-filled cells.

The more or less nonspecific mechanisms of LDL uptake by macrophages may account for the frequent presence of many lipid vacuoles in intimal cells seen as isolated elements in electron microscopy, as well as in clusters of foam cells visualized on light microscopy and in fatty streaks observed on gross inspection. Their presence would indicate that certain chemically modified forms of LDL exist, the monocyte-macrophage being unable to recognize native LDL, but only chemically modified LDL. This chemically modified LDL is, on the other hand, not recognized by endothelial and smooth muscle cells. Consequently, all modified particles of LDL would tend to be channeled to be taken up by macrophages.

Figure 65, a schematic diagram, indicates several of the mechanisms by which macrophages may accumulate lipoprotein lipids and be converted to a foam cell.[49] The existence in macrophages of β-VLDL receptors may also be involved in atherogenesis, since this existence may reflect a mechanism by which arterial foam cells are produced as a result of delivery of VLDL cholesteryl esters to macrophages. VLDL from cholesterol-fed animals and from patients with type III hypercholesterolemia and hypertriglyceridemia (or type III

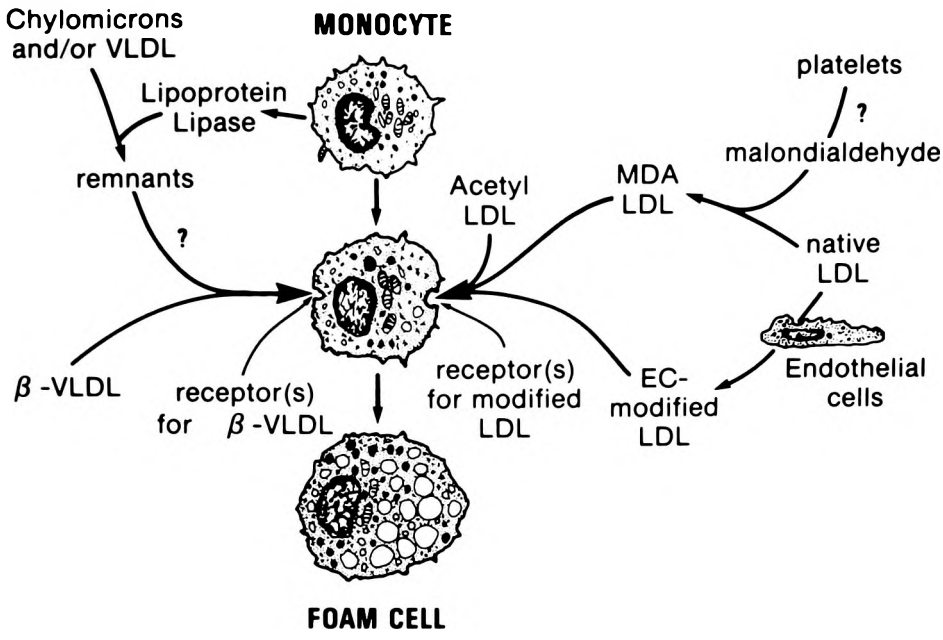


FIGURE 65. Schematic diagram indicating several of the mechanisms available to the macrophage by which it may accumulate lipoprotein lipids and be converted to a foam cell. (From Steinberg, D., *Arteriosclerosis*, 3, 283, 1983. With permission.)

hyperlipoproteinemia) have been shown to cause macrophages to accumulate lipoprotein cholesteryl esters and to assume the appearance of foam cells. The real importance in atherogenesis of the delivery of VLDL cholesteryl esters to macrophages as one of the mechanisms by which foam cells are formed has not been clearly outlined.

Macrophage recruitment in apparently normal intima is not a rare finding. Using special techniques it was possible to detect intimal macrophages in the apparently normal coronary intima of human subjects in the first month of life.[324,325] With infrequent exceptions, in the first 15 years of life, intimal macrophages occurred in the apparently unaltered intimal connective tissue as single and widely spaced cells, rather than as groups of cells. After the middle of the second decade of life, they were present in most individuals and their number per unit area in the intima increased. Their preferential location appeared to be the upper ground substance-rich and smooth muscle-poor part of the thickened coronary intima.[325]

In experimental models, macrophage recruitment involves local conditions for adequate contact with the endothelium, for adequate attachment to the endothelium and passage through this barrier; mononuclear-macrophage recruitment to the arterial intima could be enhanced approximately eightfold in mildly hypercholesterolemic animals.[326] Consistent with this finding is the enhanced monocyte recruitment in the arterial intima reported during hypercholesterolemia in a variety of species.[327] It is also worthy of note that in certain experimental models the role of the monocyte in the early development of atherosclerotic lesions was found to be minimal, if found at all.[328]

The invasion of the arterial intima by monocyte-macrophages able to give rise to lipid-laden cells may be regarded as the morphologic expression of an adequate monocyte-clearance system. The passage from monocyte to lipid-laden cell can also be considered to represent the morphologic expression of the development of an early line of resistance to lipid overload.[146,317,327,329,330]

Monocytes enter the arterial wall in the so-called prelesion stage, as deduced from gross inspection, and act as scavenger cells; many intermediate stages between the peripheral monocyte and fatty streak cells are detected. Similar intermediate stages were revealed

extravascularly in granulomata and other low turnover lesions, in which the constituent macrophages have a long life and exhibit rare mitotic figures. Based on these comparative data, it might be assumed that macrophage replication does not contribute significantly to the intimal foam cell population.

3. Subendothelial Clusters of Lipid-Filled Cells

A pathologist or experimentalist who visualizes collections of lipid-filled cells in the spleen, liver, lymph nodes, skin, or myocardium of animals submitted to cholesterol-rich diets does not record these cell collections as "lesions", but if the same animal shows lipid-filled cells in the subendothelial region of the aorta, this is recorded as "experimental atherosclerosis" and an early stage of atherosclerotic involvement. During the last decade this simplistic view was challenged by the observations which demonstrated that some of these so-called atherosclerotic lesions are, on the contrary, an early line of resistance to atherogenesis and not a pathologic change.[146,317,327]

The origin and fate of subendothelial collections of lipid-filled cells was also related to inflammatory changes.[331] Before the onset of typical atherosclerotic lesions a sticking of mononuclear cells to the endothelium can be revealed, followed by diapedesis; this was demonstrated in the pig,[146] pigeon,[332] and rat.[333,334] Involvement of chronic inflammation in experimental atherogenesis is also suggested by the fact that about 10% of the cells that invade the intima are lymphocytes.[333]

Likewise, before the onset of grossly visible atherosclerotic lesions, the occurrence of intimal insudation was frequently recorded, fibrin being an invariable component of clusters of lipid-filled cells. Its presence suggests that the primary injury to the intima is associated with increased endothelial permeability and the formation of a protein-rich exudate within the thickened intima.[335] The matrix surrounding clusters of lipid-filled cells appeared to be more susceptible to attack by muco- and proteolytic enzymes than the matrix of the intima without clusters of lipid-laden cells.[336] There is good evidence to suggest that certain plasma components which enter the intima in the insudate may produce injuries to smooth muscle cells that are reflected by progressive overload with lipid droplets. This would mean that some intimal cells may be injured by other mechanisms than hypercholesterolemia and that lipids accumulate within these cells because they are injured. It is well known that in many organs and tissues lipids accumulate within injured cells: fatty degeneration of the myocardium in anemia and steatonecrosis of the liver in chronic alcoholism are only a few examples to substantiate this view.

There are not only physiological variations in the surface charge of LDL in different subjects which could alter its atherogenic potential[337] and there are many types of smooth muscle cells and monocyte-macrophages, each with its own natural history,[338] but we must also take into consideration the changes produced by insudation in all arterial wall components, and especially metabolic changes which may promote lipid overload. When this lipid overload occurs, we must think that there are different types of subendothelial clusters of lipid-laden cells, only some of which are associated with atherosclerosis. We recorded in our human material isolated collections of lipid-laden cells: in the deep region of an apparently normal thickened intima, around areas of disintegration of the internal elastic membrane, in gelatinous lesions, in mucoid plaques, and in fibrinous remnants of partly organized thrombi. Lipid-filled cells present in the subendothelial region of an apparently unaltered vessel numbering of 25 to 30 is rapidly recorded as an independent lesion called fatty streak, but if the same number of lipid-filled cells is detected in a gelatinous lesion, their significance as fatty streak disappears and the lesion is recorded as gelatinous lesion. Likewise, if we visualized 25 to 30 lipid-filled cells in fibrinous remnants of partly organized thrombi, this is not recorded as a fatty streak, but as intramural thrombi with fatty degeneration. Finally, if we see 25 to 30 lipid-filled cells isolated in the basal region of a thickened

intima, we are tempted to neglect their presence since there is no term available to designate this presence as a lesion.

In point of fact, only the subendothelial collections of lipid-filled cells occurring on gross inspection as fatty dots and streaks have fascinated investigators for more than 100 years and have been studied extensively in experimental animals. These experimentally produced fatty streaks may be tentatively regarded in hypercholesterolemic conditions as changes in arteries equivalent to xanthomas that occur in skin and tendons. Such lesions are difficult to compare with arterial fatty streaks of human vessels which do not require hypercholesterolemia for their onset and have an universal character, particularly in large elastic arteries. It is also important to not that human fatty streaks can be found throughout the large elastic arteries from childhood onward, in the coronary arteries from adolescence onwards, in intracranial arteries from young adulthood onward, and in the renal and mesentric arteries of mature adults. Contrary to the classic opinion which erroneously maintains that fatty streaks develop only during childhood or in young subjects, we revealed their onset in the anterior cerebral, mesenteric, and renal arteries of subjects 40 to 50 years old; in the same period of life belonging to mature adulthood, fatty streaks may develop for the first time in the proximal segment of the posterior descending and first diagonal arteries of the coronary vascular bed, in the intermediate segment of the anterior descending and circumflex coronary arteries, and in many other vessels. We could detect the onset of subendothelial clusters of lipid-laden cells recorded as fatty streaks in subjects up to 50 years old. This would mean that from a morphological point of view this early lesion develops over the whole lifetime in various arterial beds and in various vessels or vascular segments belonging to the same arterial bed.

Intracellular lipid accumulation in fatty streak-like lesions is chacacterized by a net prevalence of cholesteryl esters, cholesterol esterification usually occurring *in situ*. The cholesteryl ester fatty acid composition is also characteristic, since it differs from that of blood cholesteryl esters by having a much larger oleic acid and 8,11,14-eicosatrienoic acid content and lower linoleic acid. This suggests limited availability of linoleic acid for esterification, a suggestion supported by the small amount of the 5,8,11 isomer of eicosatrienoic acid which characterizes essential fatty acid deficiency.[339] Whereas foam cells of various origin can catabolize phospholipid and triglycerides, they are not able to degrade cholesterol, but only to produce an *in situ* esterification utilizing fatty acids readily available by de novo synthesis such as oleic acid. Some investigators consider cholesterol esterification to be as a detoxification-type reaction which protects the cell from the accumulation of free cholesterol, able to alter cell membranes and to lead to cell death.[339] The intracellular conversion of free to esterified cholesterol would transform an aggressive lipid into an inactive one.

According to the location of the lipid, amount of lipid, relative proportion of connective tissue elements, and other morphologic characteristics, fatty streaks are subclassified into many types. In a study of young Americans who succumbed to sudden accidental death, it has been shown that the lesions termed fatty streaks included a variety of light microscopic features that differ in their staining characteristics.[340] Even though all were yellow and poorly demarcated, some contained most of the stainable lipid within the cytoplasm of the cells, whereas in others most of the lipid was in extracellular pools. Other studies revealed that in contrast to early fatty streaks, some lesions from individuals in their third decade of life showed the gross characteristics of fatty streaks, but electron microscopic examination showed that they had progressed beyond the stage of simple intracellular lipid accumulation. These lesions including lipid-filled cells of smooth muscle and macrophage origin or myohistiocytic foam cells,[338] might represent intermediate stages between an early and an advanced fatty streak or between a fatty streak and a fibronecrotic or fibrohyaline plaques. The conversion of fatty streaks in fibrous plaques was mainly suggested,[341] connective tissue proliferation being considered to reflect a part of the reparative process following earlier fatty change and not a primary change that initiated the lesion.[342]

As long as the lipid is intracellular and there is no connective tissue proliferation, regression of these fatty streaks without sequellae is considered possible. Xanthomas regress when cholestyramine is given to hypercholesterolemic patients, despite sustained hypercholesterolemia. Do arterial fatty streaks also diminish in size when the body sterol balance is negative? Certain investigators give an affirmative answer to this question and think that fatty streaks may be more directly influenced by the cholesterol concentration in the flowing blood than other clusters of lipid-laden cells in the body because of their proximity to the endothelium. They consider that these subendothelial clusters of lipid-filled cells have a fluctuating evolution governed by the influx and efflux of cholesterol. An interesting speculation is that if the body sterol balance were negative with unchanged plasma cholesterol and LDL levels, increased cholesteryl hydrolase activity within subendothelial lipid-laden cells might be induced with a net efflux of lipids followed by the disappearance of these clusters of lipid-filled cells. In fact, the onset of fatty streaks in experimental models could be inhibited or delayed, not only by drastic reduction of blood cholesterol levels, but also following the administration of anti-inflammatory, antimitotic, or antiplatelet agents, hormonal manipulation, and other procedures.

To sum up, the results accumulated during the last few years indicate that fatty streaks may represent true lesions, or a particular aspect of a physiological clearance mechanism, or changes of other types difficult to classify as pathologic or physiologic reactions. *The first conclusion is that we cannot consider all subendothelial lipid-laden collections as homogenous lesions which allow quantitative assessments.* The view that certain subendothelial clusters of lipid-laden cells may represent an early line of resistance to atherosclerosis rather than a pathologic response to an atherogenic agent requires further consideration. Likewise of particular interest are the factors and mechanisms which in some population groups delay or inhibit the progression of these subendothelial clusters of lipid-laden cells toward advanced lesion and in other populations favor progression. *The second conclusion is that in regression studies it is hard to assume dogmatically that the disappearance of some clusters of subendothelial lipid-laden cells is an involution of preexisting lesions.* It is possible that this disappearance reflects only the dispersion and disaggregation of preexistent lipid-filled macrophages acting as scavenger cells. An ultimate goal for prevention of coronary atherosclerosis is to delay the onset of all types of early lesions and/or to keep these early lesions small and uncomplicated. On the other hand, it would be regrettable to try to inhibit a physiological clearance mechanism able to remove abnormal lipid accumulations within the thickened arterial intimas.

The heterogeneity of cell populations present in fatty streak-like lesions[338,343-346] represents for each pathologist and investigator an important obstacle which may lead to unrealistic conclusions. Since there are different biochemical and pathophysiological mechanisms responsible for the formation of lipid-laden cells, there are also various consequences of their presence in a fatty streak-like lesion. The evidence that the arterial smooth muscle cells have a limited capacity for lipid clearance favored the general trend to search for a scavenger cell of another type, the monocyte-macrophage of the flowing blood fulfilling this role. However, even in fatty streak-like lesions in which these monocyte-macrophages predominate it is very difficult to maintain that they play only an anti-atherogenic role by removing lipids; they could also play an atherogenic role by releasing growth factors which stimulate smooth muscle cell proliferation and matrix neoformation and by other mechanisms related to inflammation and immunologic phenomena.

Wissler emphasized in a review "the enigma and diversity of the fatty streak".[340] This enigma and diversity is also emphasized in a book dealing specifically with the pathology of atherosclerosis.[347] A trap might be to consider all fatty streaks as:

1. Homogeneous lesions which can be measured after Sudan staining

2. The unique early atherosclerotic lesions, overlooking the concomitant presence of even the preexistence of fibromuscular plaques, gelatinous lesions, intimal necrotic areas, incorporated microthrombi, and intramural thrombi
3. The unique precursor of fibrous plaques without an adequate scientific proof that this conversion exists and is present in all population groups
4. The starting point of coronary heart disease, cerebrovascular disease, aortic aneurysm, etc., these apparently innocuous subendothelial collections of lipid-filled cells thus acquiring the incredible capacity to give rise to the most important part of human arterial pathology

E. Lipid Accumulation as Related to Intimal Matrix Changes

1. General Observations

In the middle of the 19th century, a concept was advanced according to which atheroma is caused primarily by nonspecific injury to the arterial wall, resulting in an inflammation characterized by an important accumulation of mucoid substances.[348] Lipid accumulation was regarded by certain investigators as a secondary phenomenon, the decisive event being intimal matrix changes.[349-351]

Starting from the 1950s, many attempts have been made to demonstrate the capacity of the intimal connective tissue of large arteries to "trap" circulating lipids by means of the glycosaminoglycan-lipoprotein complexes.[352-355]

In support of the view that the intimal matrix is involved in lipid accumulation and the onset of early atherosclerotic lesions also came the observation that species susceptible to experimental atherosclerosis usually display an important amount of intimal ground substance. In some vessels such as the aorta, the amount of glycosaminoglycans was found to be proportional to the susceptibility of the respective species to cholesterol-induced atherosclerosis.[356]

During the last decades, evidence accumulated that passage of atherogenic lipoproteins across the arterial wall might be greatly affected by subendothelial matrix properties. The capacity for hydrophilic trapping, charge characteristics, and interaction with various types of fibers permit intimal glycosaminoglycans to act like a gel-sieve ground substance. It may exert molecular sieving, steric exclusion, and electrostatic interaction which may lead to the retention of macromolecules and especially of LDL particles; moreover, it has been demonstrated that glycosaminoglycans could produce conformational changes in lipoproteins. Apparently similar glycosaminoglycans and proteoglycans act in the arterial intima as matrix builders, in joint fluid as lubricants and shock resistors, and in the eye as humor.

Many of these glycosaminoglycans and proteoglycans (this last term refers to macromolecules in which several sulfated glycosaminoglycan chains are covalently bound to a central protein core) of the intimal matrix of arteries seem to be required for endothelial and smooth muscle cells to maintain specific functions and for an adequate lipid clearance to be performed. This intimal matrix is not only a structural support for these cells, but it is also able to connect and to inform the endothelial and smooth muscle cells of events occurring in their surroundings.

Intimal glycosaminoglycans and proteoglycans exhibit an unexpected polydispersity with regard to molecular weight, carbohydrate composition of the chains, antigenic components, amino acid composition, etc. In a study in our laboratory dealing specifically with human coronary arteries,[56] we showed that the amount and macromolecular stability of each type of glycosaminoglycan seem to vary with age, anatomical branching pattern, arterial size, particular localization of the arterial segments (branching site or nonbranched region), intimal microarchitecture (one or several different sublayers), internal elastic membrane changes, etc. As a general trend, during childhood, a decrease in the histochemical reactivity of hyaluronic acid was recorded; on the other hand, we revealed the presence of hyaluronic

acid even in mature adults related to particular anatomical branching patterns, the terminal or collateral character of certain vessels, and the presence of well-developed leading edges in bifurcations and branch sites. Also as a general trend, chondroitin sulfates and dermatan sulfate increased with age in the coronary intima, but this augmentation was strongly influenced by the above-mentioned local factors. The relationship between glycosaminogylcans and elastic fiber changes was emphasized in biochemical studies and could be related to atherogenesis.[357,358]

We were deeply impressed by the fact that the extractibility of proteoglycans varies significantly with topographic sites. Chemical studies revealed that proteoglycans extracted by NaCl have a different composition than collagenase solubilized or elastase solubilized proteoglycans.[359,360] This may indicate that chondroitin sulfate-dermatan sulfate proteoglycans are mainly bound to collagen fibers and heparan sulfate to elastic fibers. Chemical results also indicate that hyaluronic acid and heparan sulfate are the most extractable and chondroitin sulfates range at the opposite end. Also of note is the observation that only 10% of the glycosaminoglycans of the nasal cartilage is extractable with 0.15 *M* saline, whereas for aortic segments 40% of the proteoglycans can be extracted under similar conditions.[360] This observation is, in our opinion, of particular importance as concerns human atherogenesis, since the coronary intima is often submitted to insudation, and the insudate, even if it appears as a simple edema, may produce matrix dissolution and disorganization. Therefore, permeability changes can largely influence the passage of macromolecules across the thickened intimas of human coronary arteries as a result of changes in matrix composition and macromolecular organization. An altered gel-sieve matrix following insudation of plasma components becomes unable to modulate LDL passage adequately by molecular sieving, steric exclusion, and electrostatic interactions and, theoretically at least, may favor lipid accumulation. Glycosminoglycan concentration as low as 0.5% or less can cause retardation of transintimal passage of lipoproteins and can also cause steric exclusion and selective ion binding. These actions are mainly exerted on atherogenic lipoproteins (LDL and VLDL) and are less obvious on anti-atherogenic lipoproteins (HDL), atherogenic lipoproteins alone being sequestered. Note also the observation that the chemical characteristics of intimal glycosaminoglycans seem to significantly influence not only the formation of lipoprotein-glycosaminoglycan complexes, but also the structural organization of LDL particles, the core cholesteryl esters of the LDL being converted from a fluid state into an organized liquid-crystalline structure.[358]

The intimal connective tissue of human arteries is made of long flexible macromolecules acting as rubber-like polymers — Brownian movement is the basis for the elementary process of diffusion. Electrostatic forces and hydrogen bonds, in addition to van der Waals forces, hold together the molecular chains of the intimal connective tissue; it is also tempting to consider that a rapid and significant decrease of the attractive molecular forces appears whenever the distance between chains is augmented. This augmentation could be produced by various types of hemodynamic and mechanical forces, especially shear stress or stretch. This may lead to changes characterized by alterations of weak van der Waals bonds. The originally tight structure of the macromolecular system changing into a loose one associated with an easier transport of the diffusing molecules. Certain investigators consider such alterations as playing an essential role in atherogenesis.[12] According to this view, injury to the endothelium is not necessary for the enhancement of transport and diffusion of molecules and particularly of cholesterol-rich lipoproteins. Alterations of certain types of intermolecular bonds seem to be the only factor required and this is usually produced by an intensified wall shear stress.

Intimal matrix may be involved in atherogenesis in a very complex manner, since:[361]

1. It regulates fluid and electrolyte balance, as well as lipid transport and metabolism across the endothelium and subendothelial area

2. It affects blood clotting and platelet aggregation
3. It influences collagen and elastic fiber formation
4. It is involved in injury, wound healing, and calcification
5. It limits the activation of complement
6. It binds and inactivates histamine and many other substances that may affect the endothelium
7. It mitigates the harmful effects of thrombin
8. It neutralizes lysosomal cationic proteins released by leukocytes
9. It reverses the effect of endothelial prostacyclin (PGI_2)
10. It inhibits the smooth muscle grow-stimulating action of PDGF
11. It enhances fibronectin-mediated macrophage phagocytosis
12. In the coronary arterial bed these highly charged macromolecules seem to attract water and produce water expulsion in relation to the intraluminal pressure variations induced by the cardiac cycle more intensely than in other vascular beds (as water is displaced, proteoglycans are forced closer together, effectively increasing the negative charge density, which in turn increases the capacity to bind LDL particles)

The involvement of the intimal matrix in atherogenesis requires taking into consideration genetic factors. Matrix changes may act in an atherogenic or antiatherogenic sense, since they may stimulate or inhibit diffusion or retention of molecules, cell proliferation, fiber neoformation, etc. The heterogeneity within each population of glycosaminoglycans is mainly due to biosynthetic differences in the core protein, as well as differences in the size, charge, and number of side chains.[362] The intimal matrix is not only submitted to a genetically determined program of biosynthesis, but is also under the influence of environmental factors, such as nutritional ones, as well as under the influence of molecular and cellular elements of the flowing blood, including hormones, growth factors, and neurotransmitters. All these influences may produce deviation from the normal program.[363] Lipid accumulation and the onset of lipid-rich lesions may be related in certain experimental models to a peculiar perturbation of this normal program of matrix biosynthesis, partly dependent on aging; likewise, certain drugs may specifically influence atherogenesis by means of their action on matrix biosynthesis.

In addition to intimal glycosaminoglycans interspersed between smooth muscle cells and fibers, there are also glycoasminoglycans located on the cell surface, especially heparan sulfate, which forms an important part of the glycocalyx of the endothelial sheet. The binding of antithrombin and α-2 macroglobulin and of lipoprotein lipase, demonstrates the involvement of these glycosaminoglycans in many important mechanisms which may be related to atherogenesis. Injected heparin-like substances, including heterogeneous mixtures of high sulfated glycosaminoglycans with a strong negative surface charge, have a high affinity for endothelium. Heparin minimizes the adherence of platelets to endothelial cells and has a general inhibiting action on platelet-collagen interaction. Likewise, it influences triglyceride lipolysis, which occurs on the vascular endothelial surface. The interaction of endothelial lipoprotein lipase with cholesterol and triglyceride-rich lipoproteins leaves remnants and LDL particles in high concentration; such remnants and particles are avidly bound by intimal cells when heparin-like substances do not exert their physiological actions. Heparin mobilizes lipoprotein lipase and displaces its activity in the flowing blood, leaving a lower concentration of atherogenic lipid-rich particles at the endothelial surface available for uptake by endothelial cells and macrophages. Hypertriglyceridemia with its secondary hypercholesterolemia and accelerated atherogenesis may result from a deficiency of endogenous intravascular heparin activity. This could lead to the assumption that *a high susceptibility to atherosclerotic involvement might be related to matrix changes associated with a deficiency of endogenous heparin supply, and that different substances which block heparin activity may act as*

atherogenic agents. An eloquent example is the LDL particle, which binds specifically to high molecular weight heparin, forming a soluble complex and concomitantly inhibiting the action of heparin.[364] This interaction of LDL particles with arterial proteoglycans is considered by certain investigators to be an additional risk factor for coronary heart disease.[365]

At the opposite end is the antiatherogenic effect of certain glycosaminoglycans present in intimal matrix and especially chondroitin-4 sulfate.[366] A crude preparation including variable amounts of heparan sulfate and dermatan sulfate was also able to reduce the atherogenic effects of LDL particles. They produce not only a lipid-clearing effect, but also inhibition of intimal smooth muscle cell proliferation and of matrix formation.

2. Selected Examples

The first material isolated as an glycosaminoglycan associated with LDL particles was a compound displaying an electrophoretic mobility lower than chondroitin sulfates[352] and able to precipitate in the presence of calcium ions.[353]

An abnormal macromolecular complex including proteoglycans and lipoproteins was obtained in vitro by adding hyperlipemic serum to the atherosclerotic intimal matrix.[354,355] A significant correlation was found between the concentration of total lipid, total cholesterol, cholesteryl esters, free cholesterol and phospholipids on the one hand, and the amount of complex formed, on the other. Studies on the thermal decomposition of proteoglycans of human aortic intima indicated that the LDL content of saline extracts of the atherosclerosis-involved areas changed parallel to the amount of proteoglycans.[355,367]

Analytical studies on the mechanism of lipid trapping by glycosaminoglycans showed that in the presence of calcium ions, the ability of these substances to form abnormal macromolecular complexes with blood lipoproteins was[368] heparin > heparan sulfate > chondroitin-6 sulfate > chondroitin-4 sulfate > dermatan sulfate.

The fact that arterial proteoglycans seem to also contain phosphate esters located primarily on the xylose residues in chondroitin sulfate chains and serine residues in the protein core suggests an additional possible interaction between intimal ground substance and lipoproteins. The development of crosslinks between the unbound sulfate groups and the phosphate radicals has been suggested to account for the progressive insolubility of glycosaminoglycan-lipoprotein complexes.[369] N-sulfate groups seem to enhance the affinity between proteoglycans and LDL particles, probably by the development of calcium bridges between these groups and phosphate radicals of phospholipids present in the lipoprotein macromolecule. If the charge profile of the native LDL particle is altered by chemical modification of the free amino groups, the ability to form ionic linkages disappears.[370]

The release of glycosaminoglycans and lipoproteins by collagenase suggests that both types of macromolecules are in addition in some way bound to collagen.[371] Association of hyaluronate as the only glycosaminoglycan in the elastase-solubilized lipoprotein fraction emphasizes the important role that hyaluronate may play in the aggregation or entrapment of atherogenic lipoproteins; part of the apo B containing lipoproteins seems to exist in human fibrous plaques in association with elastic and hyaluronate.[372]

Heparan sulfate appears less prone than other glycosaminoglycans of the arterial intimal matrix to form complexes with atherogenic lipoproteins, and once formed, these complexes occur very sensitive to changes in the ionic strength of the medium.[373]

Of late, emphasis has been put on the relationship between LDL and chondroitin sulfates and on the role of coulombic interactions in the onset of insoluble complexes.[374] Large LDL aggregates are held together by nonpolar association, the surface charge of LDL being an important modulator of the interaction with the arterial proteoglycan at low ionic strength, physiological calcium concentration, and pH. Because of its exposure at the LDL surface, sialic acid also plays a determining role in the association of LDL with the polyanionic proteoglycans. LDL from different individuals may exhibit dissimilar affinities for the li-

poprotein-complexing proteoglycan. There are high- and low-reacting LDL particles and patients with coronary heart disease present high-reacting LDL more frequently than apparently healthy controls.[365]

The rise in normal coronary arteries of dermatan sulfate and chondroitin sulfates as a function of age must be emphasized and partly related to a progression of diffuse intimal thickening. Atherosclerotic involvement of human coronary arteries accentuates this increase; the alterations found in nonatherosclerotic and atherosclerotic coronary artery wall are qualitatively similar.[375] This suggests in both aging and atherosclerotic involvement an active synthesis of connective tissue components by intimal smooth muscle cells.

If tissue fluid in gelatinous lesions is absorbed on filter paper, most of the LDL is found to be in the free fluid. This indicates that there is no evidence of specific sequestration of LDL in the intimal matrix.[197,337] There may be an increase in available extracellular space, such as that found in the loosely structural gelatinous lesion but there is no change in the mechanism of LDL uptake and retention in this atherosclerotic lesion compared with normal intima. It is highly probable that LDL particles are trapped between endothelium and the internal elastic membrane, showing a higher concentration than in the plasma. On the other hand, LDL particles present in the intimal matrix seem to be in free solution in the extracellular space, not being complexed with a compoent of the connective tissue matrix.[376,377] This may explain why most of the LDL particles in gelatinous lesions can be found in free tissue fluid. Comparison of the rates of α-2 macroglobulin and albumin to LDL in interstitial fluid and adjacent whole tissue also advocates against the formation of reversible or irreversible complexes with components of the intimal matrix.

Since in both nonatherosclerotic intima and in intima with gelatinous lesions, LDL concentration may not be significantly increase, lipid-filled cells may be absent, and smooth muscle cell proliferation cannot be associated with LDL accumulation, this suggests that LDL is not intrinsically atherogenic, focal endothelial damage is unlikely to allow more LDL to enter the intima against a twofold concentration gradient, and proliferation in early atherosclerotic lesions is not necessarily associated with an increase in LDL concentration.[197,337,376,377]

V. SUGGESTED ATHEROGENIC ROLE OF FIBRINOGEN-PLATELET-ENDOTHELIUM INTERACTIONS

A. Introductory Remarks

Rokitansky, in 1852, formulated one of the most prominent hypotheses of atherogenesis, postulating that atheroma occurs principally because of the organization of intramural thrombi. This encrustation theory considers thrombus formation as the main mechanism of atherogenesis.

By the middle of the 20th century, Duguid[378,379] emphasized the particular importance of microthrombi incorporation in atherogenesis. This view was extended and analyzed by Haust,[380] who showed that microthrombi may be found in the arteries of infants, children, adolescents, and young adults, composed of platelets and fibrin, lining on the endothelium, or endothelialized. They may be small or extend over a considerable intimal surface, the endothelialized microthrombi being progressively organized either in a thickened intima or in a developing plaque. The first endothelialized microthrombi may act as a nidus for further thrombotic deposition and the resulting lesion includes sublayers of connective tissue alternating with lipid-rich necrotic debris and with recognizably organized and unorganized thrombus. A fresh microthrombus may be superposed upon an older, less structured one, the third being even less structured, and the deepest partly organized as a fibrohyaline connective tissue. It is reasonable to assume that the degeneratvie phenomena of the unorganized thrombotic remnants play a certain role in the development of the necrotic center of plaques.[381]

It is difficult to establish the prevalence of coronary microthrombi in man, since they are not visible on gross inspection, all areas with endothelialized microthrombi being recorded as normal intima. Therefore, this prevalence is mainly based on microthrombi incidentally found on microscopic examination. In an unselected sample of the Bucharest population, including apparently healthy persons who died in traffic accidents, we were able to detect the presence of coronary microthrombi in 8% of cases 16 to 20 years old, 14% of cases 26 to 30 years old, and 22% of cases 36 to 40 years old.[6] In a study carried out on children in Tokyo, coronary microthrombi were detected in 22% of children 1 to 4 years old and 12% of children 5 to 10 years old, the mean incidence being 15.4%.[382] The incidence of microthrombi increases if isolated tissue sections are replaced by serial section and microthrombi is visualized, for instance, in seven of nine cases in subjects aged 12 to 30 years.[383] Likewise, using serial sections, pathologists demonstrated that the fibrous mass of a thrombus present in the coronary arteries may be continuous with a lesion showing all the features of an atherosclerotic plaque. This could lead to the assumption that many lesions classified as fibrous plaques on gross inspection, or as fibronecrotic or fibrohyaline plaques on light microscopic examination are in fact organized intramural coronary thrombi. This also leads to the intriguing problem of whether our experimental models based on cholesterol-rich diets have much to do with human atherogenesis.

This avascular type of organization of intramural thrombi became an interesting biological phenomenon, especially with the large use of aorto-coronary saphenous bypass grafts, since atherosclerotic plaques thought to be initiated by mural thrombi can be found in many coronary bypass vein grafts that had been *in situ* for less than 1 year.

Although all pathologists agree with the view that coronary thrombosis can be replaced by fibrohyaline connective tissue, its designation as an atherosclerotic plaque is not largely accepted. The prevalent opinion is that a mural thrombus rarely represents the starting point of an atherosclerotic plaque; in our material, using the method of similar topographic sites placed in sequence according to age, sex, and anatomical branching pattern we revealed a thrombotic origin in more than 10% of the plaques. The conversion of an intramural thrombus in an atherosclerotic plaque was far more often seen in our material than the conversion of fatty streak in an atherosclerotic plaque.

A new line of thought and investigation in the field of the thrombogenic theory of atherogenesis was opened by the important discoveries of Ross concerning the role of platelet growth factor.[131-134,137,138,184] A Rokitansky lesion has been produced by repeated or continued deposition of platelet thrombi, whereas removal or severe depletion of the blood platelets by the use of antiplatelet serum inhibited or prevented the formation of these experimentally induced arterial lesions. Studies employing labeled platelets revealed continuing uptake of platelets by the developing atherosclerotic lesions associated with fibrin and lipid deposition. The response of the arterial wall to continued or repeated injury is characterized by the formation of platelet-fibrin thrombus and lipid accumulation, even in animals without dietary lipid supplement.[186,188,191,236-239] Thrombosis is also considered crucial in the conversion of a fatty streak to an atherosclerotic plaque[384] and for an accelerated period of lesion growth during the natural history of human coronary atherosclerosis.[6]

The relationships between platelets and thrombus formation are very complex, involving activation of blood coagulation factors, deposition of fibrinogen and fibrin, and the action of hemodynamic stresses. At sites where blood flow is laminar, only a monolayer of adherent platelets forms, but at sites where blood flow is disturbed, the release of ADP and TXA_2 activate circulating platelets that aggregate with each other and with those already adhering to the vessel wall. The extrinsic coagulation pathway is simultaneously activated in and around platelet aggregates by thromboplastin released by intimal smooth muscle cells; likewise, the intrinsic pathway is activated. In experimental animals, the onset of atherosclerotic lesions may be inhibited if a platelet dysfunction exists (von Willebrand's disease)

or thrombocytopenia produced by injection of antiplatelet antibodies. Eskimos with a low platelet aggregation and long bleeding time have a lower incidence of myocardial clinical manifestation induced by coronary atherosclerosis. Theoretically, antiplatelet therapy in atherosclerosis should be able to limit intimal hyperplasia and to prevent the onset of intramural thrombi or their conversion to atherosclerotic plaques.

More than 2 decades ago it was repeatedly emphasized that *the passage from physiologic small fibrin deposits to pathologic fibrin accumulation is one of the key problems in atherogenesis.* Based on the relationship between tissue repair and fibrin deposition, maintained within physiological limits by an adequate equilibrium between coagulant activators and fibrinolytic agents, an integrative concept was proposed.[385,386] According to this concept of the hemostatic balance, fibrin is formed by the process of blood clotting and is removed by the process of fibrinolysis. The balance between the effect of these two processes would regulate the amount of fibrin deposited on the endothelium and within the thickened intima, ineffective fibrinolysis appearing as a key defect in atherogenesis. When the thromboplastic agents induce fibrin formation while the lytic agents are unable to disintegrate the newly formed fibrin networks, the onset of atherosclerotic plaques is stimulated, as well as the onset of thrombi on preexisting atherosclerotic lesions. This usually appears when the arterial intima is injured, since in the thickened intima more adequate conditions exist than in other tissue for fibrinogen-fibrin conversion and thrombus formation (an excess of both fibrinogen molecules and coagulative factors is present in the flowing blood). Note also the observation that the fibrinolytic mechanism of the arterial wall is less developed than in other tissues and may be significantly impaired during injuries. This suggests that atherogenesis might be regarded as an abnormal form of repair of intimal injuries fostered by reduced levels of plasma fibrinolytic activity, special attention being centered on the prolonged disorganizing effect of fibrin in contact with endothelium.[387] When considered from this perspective, all risk factors for coronary heart disease may be viewed as acting through a common, central initiating mechanism: an impaired fibrinolytic activity. It occurs in the presence of high LDL levels, arterial hypertension, smoking, diabetes, or obesity and can be recorded in many patients with myocardial clinical manifestations induced by coronary atherosclerosis.[388] On the other hand, high levels of plasminogen activators are associated with a reduced incidence of angina pectoris, myocardial infarction, and sudden cardiac death and this suggests that maintenance of adequate fibrinolysis is anti-atherogenic. Also of note are the observations that in the coronary circulation high shear forces are frequently associated with thrombus formation, with an abundant platelet component. In such regions, transient platelet aggregation, rather than permanent fibrin clots, would be responsible for temporary limitation of blood flow leading to life-threatening arrhythmias and focal myocardial necrosis.

Recent studies suggest that fibrin plays an important role not just in providing a scaffold for platelet aggregation, but also for smooth muscle cell proliferation and migration, macrophage attraction, and LDL particle sequestration.[389] This is compatible with the results showing that the most effective enzyme to release LDL is the fibrinolytic enzyme plasmin; fibrin seems to be specifically associated with the tight binding of LDL particles in atherosclerotic lesions.[389,390] In light of these findings, the absence of an effective system of clearing fibrin from the arterial intima may represent an atherogenic mechanism. In patients dying after myocardial infarction, a decreased fibrinolysis and fibrinolytic potential in the arterial intima was revealed and this may result in augmented intimal accumulation of fibrin.

There are many different views as to what mechanisms are more important in the thrombogenic pathway of atherosclerotic plaque formation and progression. Among these mechanisms it is not possible to overlook a deficiency of the coronary arterial wall to convert prostaglandin endoperoxide PGI_2 into an unstable metabolite which acts as a potent anti-aggregating and vasodilating factor.[391] Its importance in both thrombogenesis and atherogenesis increased when it was demonstrated that this substance is mainly produced by

endothelial cells and smooth muscle cells.[392] The capacity of both endothelial and smooth muscle cells to produce PGI_2 seems to play a central role in delaying or inhibiting atherogenesis; accordingly, hormones, drugs, and other agents which suppress PGI_2 production may stimulate thrombus formation and atherogenesis.[393]

Another interesting facet of the thrombogenic theory of atherosclerisis was suggested 10 years ago,[394] based on the existence of an endo-endothelial fibrin lining adjacent to the endothelium of arteries. Under physiological conditions, the endo-endothelial fibrin lining acts as an anticoagulant and antithrombogenic surface, the flowing blood not directly in contact with endothelial cells, but with this layer adherent to them. The endo-endothelial fibrin lining was presented as including a mixture of different forms of fibrin and its width controlled by a continuous balance between fibrin formation and disintegration.[395] Moreover, this layer seems to be a site of aggregation of fibrinogen macromolecules without thrombin cleavage. A form of clotting would take place as a growth process, layer upon layer of fibrinogen, leading to the occurrence of a gel. It is this concept of the aggregation and polymerization of fibrinogen without thrombin participation to which atherogenesis relates.[394,396] Two stages could be delineated, a first stage of atherogenesis in which a complex is formed between fibrinogen and LDL particles in the endo-endothelial fibrin lining, followed by a second stage in which gel thrombi showing a loose structure and abundant retention of LDL particles are incorporated by intimal cells giving rise to lipid-laden cells. Platelets could also be trapped in the loose or soft fibrinogen gels containing LDL (Figure 66).[394]

B. Fibrinogen and Fibrin as Atherogenic Macromolecules

Increasing evidence indicates that fibrinogen and fibrin macromolecules provide a scaffold for platelet aggregation and thrombus formation and also for smooth muscle cell proliferation and migration, attract macrophages, and sequester LDL particles.[389] Moreover, fibrin may injure endothelial cells[397] and in certain works it was possible to demonstrate that deposition of fibrin in the intima precedes LDL accumulation.[398] As damage to the endothelium is considered a key event in atherogenesis, fibrinogen and its metabolites must be considered among the most potent factors producing endothelial injury; in addition, fibrinogen and fibrin-derived peptides may induce inflammatory responses, characterized by leukocyte influx; soluble fibrin derived preparations act as direct mediators of the inflammatory endothelial cell injury.[399] Certain fibrinogen degradation products may inhibit smooth muscle cell proliferation[400] and PGI_2 synthesis.[401] In experimental animals, atherogenesis is often associated with a decrease in PGI_2 production in the arterial wall. Note the observation that in response to contact with fibrin, endothelial cells exhibit an unusual change in cellular behavior, particularly a rapid separation and migration.[402] This response did not appear with fibroblasts, epithelial cells, and a variety of other normal and neoplastic cell lines. The existence of a specific response of the arterial endothelium to fibrin deposition remains an intriguing question.

A subject of debate is also the actual design of the fibrinogen macromolecule; the most largely accepted structural model consists of three pairs of nonidentical peptide chains: $A\alpha$ (M_r 66,000), $B\beta$ (M_r 54,000), and γ (M_r 48,000). This structure $(A\alpha, B\beta, \gamma)_2$ appears organized into a central E and two peripheral D domains. It is widely believed that thrombin induces cleavage of two sets of arginyl bonds in the fibrinogen α and β chains; fibrinopeptides A and B are thus released along with the fibrin monomers having the structure $(\alpha,\beta,\gamma)_2$ which polymerize spontaneously, giving rise to fibrin networks. Fibrinopeptide A release precedes the release of fibrinopeptide B, the cleavage of peptide A being associated with the linear aggregation of the fibrin monomers, and the cleavage of fibrinopeptide B with the subsequent lateral aggreation of the preformed fibrin strands. The resulting insoluble fibrin clot is crosslinked by intermolecular covalent bonding mediated by factor XIIIA. The

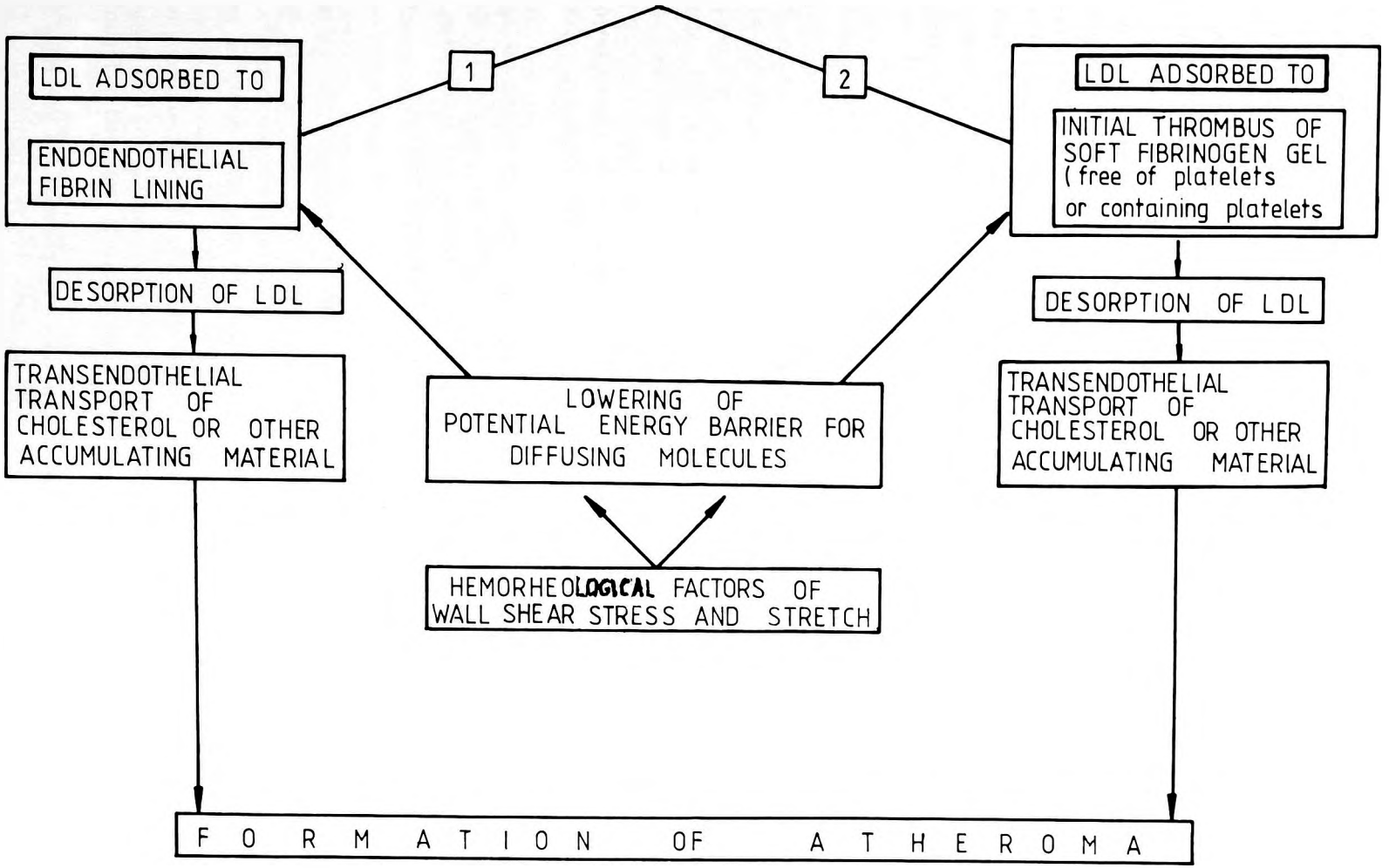

LDL ADSORBED TO
ENDOENDOTHELIAL FIBRIN LINING
1
2
LDL ADSORBED TO
INITIAL THROMBUS OF SOFT FIBRINOGEN GEL (free of platelets or containing platelets
DESORPTION OF LDL
DESORPTION OF LDL
TRANSENDOTHELIAL TRANSPORT OF CHOLESTEROL OR OTHER ACCUMULATING MATERIAL
LOWERING OF POTENTIAL ENERGY BARRIER FOR DIFFUSING MOLECULES
TRANSENDOTHELIAL TRANSPORT OF CHOLESTEROL OR OTHER ACCUMULATING MATERIAL
HEMORHEOLOGICAL FACTORS OF WALL SHEAR STRESS AND STRETCH
FORMATION OF ATHEROMA

FIGURE 66. Concept of atherosclerotic involvement based on the existence of an endoendothelial fibrin lining and two pathways of uptake of LDL particles. (From Copley, A. L., *Thromb. Res.*, 14, 246, 1979. With permission.)

maintenance of the appropriate structural conditions for thrombin-induced cleavage seems to be essential for fibrinogen-fibrin conversion, particularly crosslinking sites. Aggregates of unmodified fibrinogen may appear similar to fibrin in the electron microscope in spite of the fact that they were produced only by the precipitation of fibrinogen and not as a result of polymerization. More precise data on the macromolecular arrangement of the various domains were obtained using X-ray crystallography.[403]

As mentioned earlier, clotting of fibrinogen may appear without thrombin participation[394-396,404] (the initial phase is the aggregation of fibrinogen macromolecules, the subsequent phase fibrinogen gel clotting). Certain experiments support this aggregation of fibrinogen macromolecules without thrombin cleavage.[405] Notice the presence of substantial quantities of an insoluble form of fibrinogen differing from fibrin, and present in atherosclerotic lesions.[406] This suggests that clot formation may not be the main pathway to intraintimal deposition of this protein; the deposits might simply consist of insoluble fibrinogen with fibrinopeptide A intact, precipitated or absorbed by glycoaminoglycans and proteoglycans of the intimal matrix. Moreover, certain glycosaminoglycans may inactivate thrombin or catalyze inactivation of thrombin by the plasma inhibitor antithrombin III. A major portion of endothelium-bound thrombin appears associated with heparan sulfate of the glycocalyx and heparin may compete for thrombin with heparan sulfate sites; because of its higher affinity for thrombin, heparin may displace bound thrombin from or inhibit binding of free thrombin by the endothelium.[407]

The involvement of fibrinogen-fibrin macromolecules in atherogenesis was also related to the ability of some forms of insoluble fibrin to immobilize lipoproteins by specific complex formation or by mechanical trapping in the fibrin mesh.[389] Whatever the mechanism, it suggests a synergism between plasma fibrinogen and lipoprotein in the accumulation of cholesterol in early atherosclerotic lesions. Moreover, certain lipoproteins may influence both fibrin formation and degradation. Thus, for instance, the major proteins of HDL, apo A-I and apo A-II and certain minor constituents common to HDL and VLDL participate in fibrinolysis, enhancing urokinase-induced plasminogen activation.[408] Since apo A-I and apo A-II can be synthesized by arterial endothelium, which also produces the plasminogen activator, these two secretory products may have a synergistic role and their inhibition seems to act as an atherogenic condition.

A decrease in fibrinolytic activity was recorded by many investigators in women taking oral contraceptive pills. The decrease led to enhanced blood coagulability, associated with a low content of plasminogen activators, enhanced platelet adhesiveness, and aggregation, a rise in clotting factors VII and X, and other changes of an atherogenic character.

C. Platelet Involvement in Atherogenesis

While it is clear that fibrinogen-independent pathways of platelet aggregation exists, it is equally clear that fibrinogen-dependent platelet aggregation represents a major pathway in atherogenesis.[409] This major pathway requires the binding of circulating fibrinogen to specific receptors on the platelet membrane, the adherence of platelets to each other occurring as long as fibrinogen receptors are available on the surface of platelets. The interaction necessitates platelet stimulation and the induction of receptor sites, since fibrinogen receptors are latent on unstimulated platelets, thus preventing their spontaneous aggregation by plasma fibrinogen. A hypothetical model of platelet-fibrinogen interactions is presented in Figure 67.[410]

The dissolution of fibrin clots in vivo is mainly produced by plasmin and generates a series of degradation products which may interact with platelets. The two peripheral or D domains of the fibrinogen macromolecule contain a portion of each constituent chain. Cleavage at this portion results in the generation of a family of degradation products M_r 11,270 to 240,000, designated as fragment X, whereas cleavage of fragment X gives rise to fragment

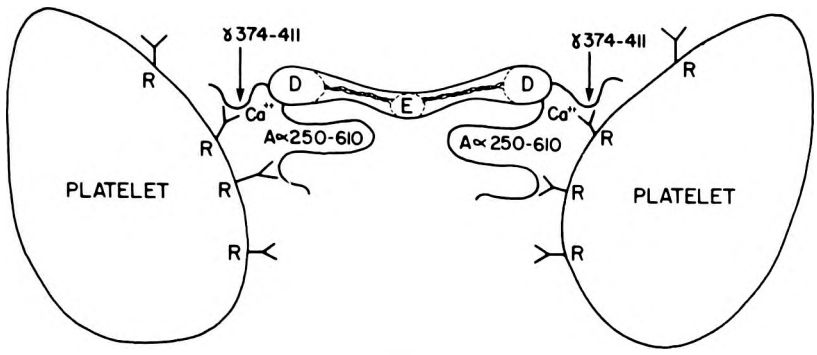


FIGURE 67. Hypothetical model of platelet-fibrinogen interactions. R indicates either high or low affinity fibrinogen receptors. The size of the platelets and the fibrinogen molecules do not reflect their real dimensions. (From Niewiarowski, S. et al., *Ann. N.Y. Acad. Sci.*, Suppl. 408, 536, 1983. With permission.)

Y (M_r 165,000 to 155,000) which is comprised of a D and E domain, and fragment D (M_r 100,000 to 80,000). The native structure and conformation of the fibrinogen macromolecule seems to be very important for platelet aggregation: the asymmetric cleavage of fragment X to generate fragments Y and D is associated with a complete loss of capacity to support platelet aggregation.[409]

Platelets respond to a number of stimuli by changing shape and by aggregating into large clumps, a process associated with the exposure of previously cryptic receptors for fibrinogen and the expression of procoagulant activity. The ability of ADP to expose fibrinogen receptors on the platelet surface has been well documented as was the role of fibrinogen as a cofactor for the aggregation of platelets by ADP. Binding of fibrinogen has also been demonstrated following platelet stimulation by thrombin, arachidonic acid, prostaglandin endoperoxides, thromboxanes, and epinephrine. Fibrinogen also appeared bound to platelet receptors during collagen-induced aggregation and this binding was mainly attributed to the release of intracellular ADP.

Emphasis is also placed on the control of the formation and dissociation of the glycoprotein IIb and IIIa complex which constitutes the fibrinogen receptor on the platelet membrane. The fibrinogen binding is specific and saturable; it does not require the generation of additional intracellular signals or the production of chemical messengers. Likewise, binding does not imply conversion to fibrin of fibrinogen macromolecules, so that fibrinogen-platelet interaction is a function of the native fibrinogen, requiring only divalent ions, such as calcium or magnesium. Fibrinogen binding mediated by various stimuli (thrombin, collagen, epinephrine, arachidonate, certain prostaglandin derivatives) seems to occur through the same mechanisms and involves the same binding sites: the glycoprotein IIb and IIIa complex, a major platelet membrane glycoprotein constituent.

The following steps have been suggested to occur in platelet-fibrinogen interaction:[409] encounter of the platelet with stimulus and the triggering of initial events, induction of the fibrinogen receptor on the cell surface, reversible binding of fibrinogen to its receptor, and stabilization of the platelet-fibrinogen interaction. Whereas platelet aggregation appears strongly dependent on the availability of fibrinogen receptors, fibrinogen causing aggregation by binding adjacent platelets together, an excess of fibrinogen may block platelet aggregation.[410] Among substances released by platelets, thrombospondin and fibronectin appear to be mainly involved in platelet-fibrinogen interactions. Thrombospondin facilitates platelet interaction with fibrinogen and fibronectin with fibrinogen and collagen. Fibronectin possessing binding sites for both fibrinogen and collagen might be regarded as the mediator in the binding of the two proteins and deeply involved in atherogenesis.[411] It seems well

established that activated factor XIII is able to crosslink fibronectin in fibrin and fibrinogen. In addition, thrombospondin released from platelets binds to their surface and could be involved in adherence to fibronectin. A hypothesis was advanced that one of the physiological functions of fibronectin is to stimulate the formation of a pseudoendothelial carpet of unadhesive spread platelets in deendothelialized zones of the arterial wall by binding to these zones.[412] In atherosclerotic plaques fibronectin is concentrated in the cap of the lesion.[413]

Platelet involvement in atherogenesis is also related to the fact that they possess a number of coagulant activities leading to fibrinogen-fibrin conversion and/or direct fibrin formation. The platelet membrane, during shape changes following activation, becomes a catalytic surface which promotes the formation of thrombin in the presence of activated factor X, factor V, and calcium ions. During activation, platelets are the site of a local generation of thrombin and this can stimulate other platelets to aggregate and the results in the formation of the platelet thrombus.[414] Thrombin can induce platelet aggregation through pathways that are independent of the arachidonate pathway and released ADP. Collagen also causes the release of ADP from platelets independent of the activation of the arachidonate pathway.[415]

The platelet mass that forms serves as a focus for the acceleration of the intrinsic pathway of coagulation through the interaction of factors VIII and IX and of factors V and X that take place in association with the platelet membrane phospholipids. The end result is the formation of fibrin networks around the platelet aggregates exhibiting an important stabilizing effect. The bases of many thrombi present in the coronary arteries of middle-aged adults have large amounts of fibrin, whereas the upper part within the lumen is composed of fibrin, platelets, and white cells. The light microscopic feature of this type of thrombus frequently seen in the coronary arterial bed is compatible with the generation of thrombin through the activation of blood coagulation and fibrin formation as initiating event, followed by platelet accumulation and stabilization by the fibrin network.

Platelet involvement in atherogenesis and coronary heart disease may be also related to the fact that α-adrenergic receptors are present on the platelet surface and this could explain why epinephrine and norepinephrine induce platelet aggregation and potentiate aggregation produced by ADP. These effects may be prevented by nonselective and selective α-adrenergic antagonists. The existence of platelet α-adrenergic receptors might explain the atherogenic role attributed to emotional stress and especially its capacity to precipitate sudden cardiac death. Increased catecholamine secretion during emotional stress may activate platelets directly and this may be followed by the onset of intramural thrombi and thrombus disintegration with peripheral embolization. In certain works, a clear demonstration was made that platelet activation and secretion occurred in association with an increase in plasma catacholamine levels.[417] Platelet abnormalities occurring as a higher sensitivity to catecholamine aggregation are more frequently found in patients with transient ischemic attacks; these attacks probably have a thromboembolic basis and drugs which interfere with platelet aggregation could be useful in these pathological conditions. According to this view, the clumping of platelets constitutes the primary event in coronary atherogenesis, a view supported by the results of certain experimental models.

In our material, the onset of intramural thrombi and thrombi incorporation into the thickened intima as the first manifestation of atherosclerotic involvement appeared less related to the presence of platelet aggregates then to the local accumulation of fibrin. This was presumably a function of the concentration of fibrinogen, the rate of conversion of fibrinogen to fibrin, and the rate of degradation of fibrin by plasmin and other proteolytic agents. The use of only ordinary preparations could not provide an adequate answer if the clumping of platelets plays a major role in human coronary atherogenesis.

The involvement of platelets in atherogenesis cannot be limited to their capacity to produce microthrombi and intramural large thrombi — of particular importance is the release of their stored granules.

The first response of platelets to aggregating agents is a change in shape followed by reversible or irreversible aggregation. The second burst of aggregation is associated with release of constituents from dense granules, lysosomes, and α granules, as well as with the arachidonic acid metabolism activation.

The nonmetabolic pool of adenine nucleotides, 5-hydroxytryptamihne, pyrophosphate and calcium ions are stored in the dense granules. The α granules contain numerous proteins with biological activity; some of these secreted α grnaules contain proteins that are platelet specific (platelet factor 4, β-thromboglobulin, PDGF, thrombospondin), while others are similar to plasma proteins (fibrinogen, fibronectin, von Willebrand factor, von Willebrand antigen II, factor V, albumin, α-$_2$-antiplasmin, α_2-macroglubulin, plasminogen, α_1-antitrypsin, histidine-rich glycoprotein). Owing to this complex secretion and release, platelet granule constituents may be considered to be involved in coagulation, arterial wall repair, and atherosclerotic involvement.

Of particular importance in atherogenesis are the influences exerted by these platelet granule constituents on endothelial cells and the subendothelial zone, since it is in this part of the artery wall to which platelets attach and the coagulation system is activated. Platelets adhere to several forms of fibrillar collagen which induced the release of the platelet granules; this complex interaction is followed by the stimulation of the arachidonate pathway and liberation of antiheparin platelet factor 4 and factor XII. The contact with collagen activates factors XI and the coagulation cascade is initiated.

It is worth remembering that eicosanoids are not stored in cells but are released immediately after biosynthesis and are capable of producing important variations in the coronary arterial tone, particularly of small intramyocardial vessels. Arachidonic acid is present in the phospholipids of the cell membrane and is derived from the diet, being synthesized by chain elongation and desaturation of dietary linoleic acid. The cyclooxygenase enzyme complex oxygenates arachidonic acid to the prostaglandin endoperoxides PGG_2 and PGH_2, which are then converted to the various prostaglandins and thromboxanes. Platelet stimulation by thrombin, ADP, calcium, and epinephrine is associated with activation of membrane phospholipase and of the cyclooxygenase pathway with the formation of various metabolites. Platelets can also metabolize exogenous arachidonate to produce PGH_2 and TXA_2, both of which are thought to be stimulants of platelet aggregation. On the other hand, platelets produce PGI_2 which inhibits platelet aggregation by raising intracellular cAMP. When inhibitors of one pathway of arachidonate metabolism are used, metabolites from other pathways will increase (inhibition of cyclooxygenase may cause augmentation in substances involving an increased activity of the lipooxygenase pathway).

Since TXA_2 is important in platelet aggregation and the release of the contents of platelet granules, certain authors think that in circumstances in which the production of TXA_2 is enhanced, platelet aggregation and thrombosis will be more extensive and coronary atherosclerotic involvement more severe. Beginning with this view, many attempts have been made to inhibit platelet function through cyclooxygenase inhibition, thromboxane synthetase inhibition, and the use of specific thromboxane TXA_2 antagonists or PGI_2 agonists. Specific receptor agonists or antagonists might have a theoretical advantage over inhibitors of arachidonic acid metabolism because they should not directly alter other aspects of arachidonate metabolism. Attempts have also been made to find a drug or an aspirin dosage that selectively interferes with the platelet enzymes without or only minimally affecting the artery wall and particularly the endothelial cyclooxygenase. Such a drug would be able to inhibit TXA_2 production by platelets without inhibiting PGI_2 formation in endothelial cells.[418,419] Patients with myocardial clinical manifestations induced by coronary atherosclerosis have been found to present an increased sensitivity to thromboxane and a reduced sensitivity to PGI_2.[420] Platelets from patients with type II A hypercholesterolemia seem to be more sensitive to aggregation induced by thromboxane and also show an altered response and synthesis of

arachidonic acid metabolites.[421] Aggregation of platelets from these patients requires more PGI_2 to be inhibited than that of platelets from normal subjects.[422] An association seems to exist between hyperlipidemia with enhanced platelet sensitivity to aggregating agents and increased synthesis of TXA_2 by platelets. All these results and contributions concerning platelets physiology and pathophysiology are very important, but their real value in human coronary atherogenesis remains to be demonstrated.

Diets enriched in saturated fats are associated with platelet hyperactivity and altered metabolism which favor a prothrombotic state in populations consuming high saturated fats.[423] This may be related to epidemiological studies which have shown that diets rich in cholesterol and saturated fat are associated with an increased incidence of myocardial clinical manifestations of coronary thrombosis. A view was advanced that the low incidence of such manifestations in populations consuming diets rich in linoleic acid may reflect a reduced conversion of arachidonic acid to TXA_2. Greenland Eskimos, who consume a diet rich in eicosapentaenoic acid and poor in arachidonic and linoleic acid, have a bleeding diathesis and seldom develop myocardial clinical manifestations induced by coronary atherosclerosis; eicosapentaenoic acid acts as an agent to inhibit platelet aggregation.[424] In subjects consuming diets enriched with eicosapentaenoic acid, platelets respond less strongly to stimulation and this might be considered as an anti-atherogenic condition. Eicosapentaenoic acid reduces platelet aggregation due to a competitive inhibition of the conversion of arachidonic acid into TXA_2; it also blocks TXA_2 receptors in the platelet membrane. On the other hand, it does not inhibit endothelial cell PGI_2 synthesis, the effect of incorporating eicosapentaenoic acid into structural lipids being a shift in the hemostatic balance in an antithrombotic direction.[425]

From several lines of evidence there is now little doubt that saturated dietary fats influence platelet behavior and may cause thrombosis and atherosclerotic involvement, whereas polyunsaturated fats may have an opposite effect. Different dietary fats could alter platelet lipid composition, platelet aggregation and adhesion, and prostaglandin metabolism. Significant variations in dietary levels of polyunsaturated fatty acids may profoundly affect eicosanoid synthesis in platelets either by changes in the tissue levels of the fatty acid precursors or by affecting the enzymatic system.[426] The observation of a low incidence of myocardial infarction and sudden cardiac death in populations eating appreciable amounts of eicosapentaenoic acid has promoted a number of studies on the possible anti-atherogenic effect of this fatty acid. The results show that it may produce an inhibitory activity on the platelet cyclooxygenase system and may reduce the synthesis of thromboxanes.[426] On the other hand, the hypersensitivity of platelets may also be associated with a modification of the cell membrane induced by an altered cholesterol-phospholipid content. The incubation of normal platelets with cholesterol-rich liposomes resulted in increased amounts of released arachidonic acid by thrombin-stimulated platelets and in a significantly higher thromboxane synthesis, as compared with platelets incubated with cholesterol-poor liposomes.[427] In essence, both the diet and the lipoprotein pattern have been shown to affect the aggregation of platelets directly; in addition scanning electron microscopy (SEM) revealed important platelet changes in patients with myocardial clinical manifestations induced by coronary atherosclerosis.[428]

The role of platelets in atherogenesis has received strong support as a result of many observations showing that the incorporation of clusters of platelets takes place within the thickened arterial intima and that this incorporation constitutes the chief factor in determining the clinical significance of an individual atherosclerotic lesion.[429,430] Patients have presented with prolonged thrombocytsis and with many intramural thrombi in different arterial beds causing infarcts.[431] Thromboembolic events in the coronary arteries are not uncommon in patients with congenital clotting deficiencies and the existence of these "experiments of nature" underscores the little-recognized phenomenon of uncontrolled thrombosis.[432]

The long-standing debate about the extent and the importance to which platelets and fibrin initiate atherogenesis and contribute to the development of early atherosclerotic lesions has

received a fresh stimulus from the demonstration that organizing thrombi can acquire cell populations with monoclonal characteristics.[173]

In conclusion, starting from the results of many clinical and experimental data, it might be presumed that in areas of endothelial injury of the coronary arterial tree platelets accumulate and spread upon the altered endothelial surface. There is a gradual build-up of platelet mass with some fibrin formation, and if the endothelial injury continues, a markedly enhanced thrombogenic response ensures with more intense fibrin formation leading to the onset of microthrombi or intramural thrombi. The causes of this enhancement of thrombogenic response are complex, related to changes in the flow pattern, intimal microarchitecture, proteoglycan composition, release of growth factors, stimulation of coagulation cascade, etc. Platelets themselves represent a source for the phospholipids needed for full activation of the coagulation system, and the platelet surface may serve to bind at multiple sites factors involved in thrombus formation, particularly fibrinogen macromolecules. The presence of fibrinogen and fibrin within the platelet nidus adds stability to the developing thrombus, since in the absence of this stability platelet microthrombi are potential sources of embolization. They can travel through the coronary arterial tree until they come to a small branch which is too narrow to allow further passage; in the respective branch platelet emboli will lodge, occlude the vessel, or disintegrate.

Unfortunately, the human pathologist can very rarely detect such dynamic changes, the prevailing aspects being those of fibrin accumulation. The initial intramural thrombus or incorporated microthrombi very often seem to be only a mass of fibrin in which platelets may or may not be trapped. The progressive growth of these small thrombi in the coronary arteries appears in the post-mortem material to be dependent on additional fibrin deposition, rather than on platelet accumulation. A crucial point essentially remains to be demonstrated based on more adequate material and methods; are the early atherosclerotic lesions of thrombotic origin derived mainly from the incorporation of platelet microthrombi or fibrin microthrombi, or from fibrinogen macromolecules which pass across the endothelium as plasma components? From the many reports on this subject, it is evident that local conditions exist which permit fibrinogen and fibrin to accumulate without the presence of platelet microthrombi or polymerized fibrin bands of microthrombi. Of interest are the experimental studies which support the hypothesis that a thrombus may be organized by cells derived from the circulating blood.[433] In our material we have observed both platelet- and fibrin-rich microthrombi progressively incorporated and organized in developing plaques. The use of successive microscopic observations of similar topographic sites placed in sequence according to age, sex, and anatomical branching pattern revealed that the first endothelialized microthrombus may act as a starting point for further thrombotic deposition and plaque formation. Especially at branch sites of the coronary arterial tree we revealed many lipid-free fibrohyaline plaques which exhibited layered features, suggesting several episodes of fibrin accumulation.

D. Endothelium and Thrombogenesis

Endothelial cells have both procoagulant and anticoagulant properties. They are deeply involved in coagulation since they produce plasminogen activator, plasminogen activator inhibitor, factor V, thrombospondin, and von Willebrand's factor; factors IX and X also bind to endothelial cells, as do protein C and antithrombin III.

Endothelial cells also metabolize arachidonate, mainly through the cyclooxygenase pathway and are able to produce heparin-like proteoglycans. The proteoglycans and glycoproteins present on the endothelial luminal surface have a composition and distribution that may be related to various endothelial cell functions such as transport, permeability, anticoagulant surface and antithrombogenic surface, metabolism of circulating mediators, etc. Cultured endothelial cells can release potent anticoagulant heparan sulfate and its release seems to be

potentiated by activated clotting factors; thrombin, for instance, being able to determine the release of heparan sulfate from endothelial cells.[434]

Important amounts of sialic acid were also detected on vascular surfaces exposed to the flowing blood, providing extraordinarily high negative charge densities.[435] When the endothelial surface is selectively desialated, the nonadherence of platelets and the nonthrombogenicity of the endothelial surface might disappear. In addition, an increased uptake of circulating LDL and fibrinogen by the arterial walls after removal of sialic acid from the endothelial surface was recorded.[436,437]

In physiological conditions endothelial cells form a continuous luminal monolayer that creates a thromboresistant barrier between the flowing blood and the arterial wall. Many investigators consider the endothelial integrity central to their concepts on atherogenesis and assume that atherosclerotic lesions might occur only when the integrity of the nonthrombogenic endothelium is lost. Endothelial integrity has been implicated as a main mechanism in atherogenesis based only on experimental data which revealed a direct relationship between the frequency, magnitude, and duration of endothelial damage and subsequent development of atherosclerotic lesions.

In many experimentally induced lesions after endothelial denudation, extensive endothelial damage was followed by exposure of subendothelial components of large arterial segments to the flowing blood. In these experimental models platelets appeared unable to adhere to the arterial wall without an available thrombogenic surface. The practical conclusion of the results of these investigations is that the possible avenues for prevention and control of atherogenesis may lie in maintaining endothelial integrity. In other studies, endothelial injury was not necessary for the onset of atherosclerotic lesions. All these data refer to experimental models which sometimes equate endothelium, which is a specialized structure of the arterial wall with cultured endothelial cells.

It seems logical to accept the view that the major risk factors for coronary heart disease may cause endothelial injury. Cigarette smoking could alter endothelium particularly by means of carbon monoxide, arterial hypertension by means of intensified hemodynamic stresses, and hypercholesterolemia by means of a direct action of LDL particles on endothelial cells. The end result of these complex and repeated injuries might be the occurrence of an intramural thrombus which gives rise to an atherosclerotic plaque. The endothelial injuries produced by risk factors for coronary heart disease would be aggravated by immunological reactions, bacterial endotoxins, hypoxia, shock, high levels of catecholamines and other factors, and mechanisms which may lead to intimal edema, accumulation of inflammatory cells, proliferation of smooth muscle cells, and particularly the occurrence of intramural thrombi.

Two mechanisms appear mainly involved in the onset of these intramural thrombi. One mechanism is largely platelet-dependent and able to be inhibited by drugs such as aspirin and another mechanism with a significant coagulation component. When this latter mechanism prevails, endothelial injury is associated with inhibition of the fibrinolytic system. This system comprises a proenzyme, plasminogen, which can be activated to plasmin that will degrade fibrin by several types of plasminogen activators. Efficient thrombolysis seems to be regulated via adsorption of the tissue-type plasminogen activator and plasminogen on the fibrin surface and the *in situ* generation of plasmin. Inhibition of the fibrinolytic system during endothelial injury may occur at the level of plasmin or at the level of the activators. Activating agents, such as tissue-type plasminogen activators and urokinase, promote the conversion of plasminogen to plasmin, thus initiating fibrinolysis. Apo A-I and apo A-II, major proteins of HDL, activate fibrinolysis enhancing urokinase-induced plasminogen activation.[227] These apo A-I and apo A-II apoproteins can be synthesized by human endothelial cells,[226] and this elaboration might be inhibited during endothelial injury. Since endothelial cells also produce plasminogen activator, it is tempting to suggest that these two secretory

activities may have a synergistic role and their inhibition or disappearance may favor thrombus formation.

It is worth remembering that disturbances in the balance between the production of TXA_2 by the platelets and of PGI_2 by the endothelial cells were deeply involved in thrombogenesis.[391,392,438-440] The endothelial cells synthesize PGI_2, not only from its endogeneous precursors, but also from prostaglandin endoperoxides released by platelets. The anti-aggregating and antithrombotic activity of endothelial cells is mainly related to their capacity to release PGI_2, the most potent endogenous inhibitor of platelet aggregation yet discovered. In experimental models, PGI_2 released by endothelial cells is able not only to inhibit thrombus formation, but also to protect against sudden death due to platelet clumping induced by intravenous arachidonic acid in rabbits.[441] Intravenous infusion of PGI_2 was also given to patients with coronary heart disease; the effects obtained were considered similar to those of short-acting nitrates, since PGI_2 is not only a strong inhibitor of platelet aggregation, but also a vasodilator.[439,440] According to certain views, PGI_2 in pharmacological doses might represent an important therapeutic approach under pathologic conditions characterized by a documented increase in the consumption of platelets: extracorporeal circulation, renal transplants, peripheral vascular disease, pulmonary hypertension, and coronary heart disease.

PGI_2 produced by endothelial cells is an unstable compound and its synthesis under normal conditions is low or very low. An increase of this synthesis is considered to indicate changes in the interactions between platelets and endothelial cells.[420] An overresponse of endothelial cells in terms of PGI_2 synthesis was recorded in areas with developing atherosclerotic plaques. β-Receptor blocking drugs such aspindolol and propranolol stimulate PGI_2 synthesis in the coronary arterial wall, their effects being related to both adrenolytic and antiplatelet actions.[442]

Increasing evidence indicates that in pathological conditions associated with augmented platelet aggregability, the sensitivity of platelets to the inhibitory effect of PGI_2 is reduced. Analytical studies revealed that PGI_2 inhibits platelet aggregation by stimulating adenylate cyclase, leading to an increase in cAMP levels in the platelets that persist for some time. PGI_2 not only increases adenylate cyclase activity by acting on distinct receptors on the platelet membrane, but also enhances calcium sequestration and exerts an inhibitor effect on platelet phospholipase and platelet cyclooxygenase, both related to its ability to increase cAMP. Thus, by inhibiting several steps in the activation of arachidonic acid metabolic cascade, PGI_2 exerts an overall control of platelet aggregability. The fact that PGI_2 inhibits platelet aggregation (platelet-platelet interaction) at much lower concentrations than those needed to inhibit adhesion (platelet-collagen interactions) suggests that PGI_2 allows platelets to stick to the arterial wall and interact with it, while at the same time preventing or limiting thrombus formation.[443] In certain experimental models this capacity of PGI_2 to prevent thrombus formation when applied locally in low concentrations was clearly demonstrated, but the duration of this effect is very short.

Attention for some time has focused on the possible interactons between endothelial PGI_2 and blood lipoproteins. Both LDL and HDL may infleunce PGI_2 production by the endothelium, LDL showing an inhibitory effect, HDL a stimulating effect; it might be of interest to remember that this production seems to be under a negative feedback control.[444] Incubation of endothelia cells with HDL may protect against LDL-induced injury and may augment the synthesis of PGI_2. This synthesis might be also influenced by the blood levels of atherogenic lipoproteins, LDL exerting a direct effect on platelet aggregation; in some investigations, PGI_2 appeared less effective in inhibiting platelet aggregation when increased amounts of LDL were present.[445,446]

It has been suggested that smooth muscle cell proliferation in atherosclerotic plaques might be a consequent of PGI_2 inhibition by lipid peroxides.[443] To correct this deficiency, the use of synthetic PGI_2, of stimulators of endogenous PGI_2 production, and of antioxidants was attempted.[447]

A number of promising studies are underway which try to demonstrate that the exhaustion of PGI_2 synthesis is deeply implicated in atherogenesis and that this exhaustion appears before any detectable morphological alteration. PGI_2 production by atherosclerotic lesions was found to be significantly lower than that of the normal endothelial area, but no differences were recorded between early and advanced lesions.[448]

Failure of the endothelium to produce PGI_2 could also reduce the ability of the coronary artery vessels to relax when they are stimulated to contract.

The discovery that the endothelial cells generate an unstable PGI_2 with properties diametrically opposite to those of platelet TXA_2 has promoted great speculation on the role in thrombogenesis and atherogenesis of variations of PGI_2 vs. thromboxane ratio. A decreased sensitivity of platelets to PGI_2 was detected in patients with angina pectoris,[420] and a prevalence of thromboxane was mainly recorded in unstable angina pectoris.[449] Enhanced thromboxane activity may either precede or follow myocardial ischemia and could be a factor in the initiation and development of the ischemic episode. This leads to the large use of aspirin, since acetylsalicylic acid given in small doses may inhibit the production of thromboxane without affecting that of PGI_2. Even if it reduces PGI_2 synthesis, the drug is beneficial as an antithrombotic agent, possibly because some of its activity is not related to the inhibition of cyclooxygenase.[450] In essence, there is ample experimental and clinical evidence to suggest that an imbalance between thromboxanes produced by platelets and PGI_2 produced by endothelial cells might contribute to atherogenesis, thrombogenesis, and coronary flow abnormalities due to vasospasm, platelet plugs at sites of coronary obstruction, or transient ischemic events in myocardium.[451]

Worthy of remark is the assumption that the repulsion of platelets by endothelial cells is an active process, involving continuous synthesis of PGI_2. This view is not in keeping with the concept that the prostaglandins are formed in short-lived bursts in response to specific cell surface stimuli and act as local hormones or autocoids. One of the few examples of systemic effects of prostaglandins refers to PGI_2 produced by the uterus, acting as a circulating vasodepressor in pregnant dogs.[452]

Another much debated question related to the suggested role of endothelial injury in atherogenesis and thrombogenesis is the importance of intracellular peroxidation. Peroxidation of polyunsaturated lipids and the formation of free radicals may damage endothelial cells, particularly endothelial cell membranes. Under normal conditions acting against free radical formation are the enzymes that may transform toxic lipid hyperperoxides into harmless hydroxides. Animal experiments showed that if this defense mechanism fails (vitamin E deficiency), this may be followed by endothelial injury and the development of platelet thrombi.[453] In man, oxidant-antioxidant imbalance may determine liver necrosis, kidney degeneration, heart and vessel wall alteration, and other severe pathological changes. Glutathione peroxidase seems to function synergistically with vitamin E in protecting cellular membranes from lipid peroxidation, the oxidation of polyunsaturated lipids leading to the formation of highly unstable free radical intermediates. Vitamin E seems to have a protective effect on PGI_2 production and its use in the prevention of thromboembolic disease was suggested.

PGI_2 synthesized by endothelial cells might be involved in atherogenesis and thrombogenesis, not only by its influence on platelet metabolism and behavior, but also in an indirect manner by its influence on coronary artery tone. Both atherogenesis and thrombogenesis are inhibited by the vasodilator action of PGI_2, this PGI_2 is also able to modulate the coronary artery wall response to norepinephrine, serotonin, angiotensin, etc. According to the results obtained in certain experimental models, one of the main roles of prostaglandins present in the coronary circulation is to reduce sympathetically mediated vasoconstriction. This is in agreement with the finding that stimulated endothelial cells release a labile oxidation product of arachidonic acid that relaxes medial smooth muscle cells, acting as an endothelium-

dependent relaxing factor. It is thus tempting to relate the consequences of endothelial injuries not only to atherogenesis, but also to the pathogenesis of coronary heart disease.

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Chapter 4

NATURAL HISTORY OF CORONARY ATHEROSCLEROSIS AS RELATED TO AGE

I. INTRODUCTORY REMARKS

A. Natural History, A Very Complex Study

The natural history of human coronary atherosclerosis includes a series of cascade interactions involving genetic and environmental factors, components of blood and arterial wall, particular hemodynamic stresses, and other as yet unknown atherogenic agents. Theoretically, an adequate study of the natural history of human coronary atherosclerosis requires delineation of at least five major successive stages:

1. Prelesion period
2. Period of onset of early lesions
3. Period of onset of early lesions associated with progression of some of these lesions toward advanced forms
4. Occurrence of stenotic or obstructive lesions able to give rise to silent myocardial ischemia
5. Events which determine myocardial clinical manifestations

An adequate study on the natural history of coronary atherosclerosis also implies analyzing only "pure cases", namely subjects without associated diseases and which were not submitted to preventive measures, treatment, or surgical interventions. Our material corresponds to a certain extent to this requirement since it includes apparently healthy persons who died in traffic accidents and one might presume that they exhibited a natural history of coronary atherosclerosis similar to that existing in living subjects with a normal lifestyle.

Preventive measures and particularly medical and surgical treatments may influence the natural history of coronary atherosclerosis. In the U.S., for instance, more than 100,000 patients per year currently undergo myocardial revascularization surgery and must be included in a modified form or type of the natural history of coronary atherosclerosis. Moreover, a particular natural history of vein graft atherosclerosis was recently delineated, intricated with that of the coronary arteries.[1]

During the last few decades the natural history of coronary atherosclerosis was, to a certain extent, modified by the substantial augmentation of the older population. This population is expected to have the highest incidence and prevalence of the advanced forms of coronary atherosclerosis and of myocardial clinical manifestations induced by coronary stenotic and occlusive plaques. In addition, the aging of the population leads to an increase of cases in the preclinical stage of coronary heart disease and of cases with silent myocardial ischemia. The earlier cardiologists, lacking in vivo coronary angiography, were inclined to consider the existence of a sharp delineation between healthy persons and coronary patients. They systematically overlooked cases with silent myocardial ischemia; during the past 2 decades increased exposure to diagnosis by means of coronary angiography has dramatically changed some of the classical views.

Given the plurietiologic nature of coronary atherosclerosis and the important number of pathogenetic mechanisms involved, a realistic view of the natural history of coronary atherosclerosis requires a level of complexity and integration that is far greater than that largely accepted. This is the reason why the natural history of atherosclerosis is still "a subject of considerable uncertainity, vigorous debate and active investigation".[2]

The main aim of this active investigation is to try to explain why and how an inherited life span comes to an end manifested clinically as sudden cardiac death or myocardial infarction, or comes to an end manifested clinically as a noncardiac and noncoronary cause in spite of the presence of a severe atherosclerotic involvement. Whereas the study of the characteristics of patients with coronary heart disease induced by coronary atherosclerosis was and continues to be a subject of innumerable investigations, the existence of an asymptomatic coronary atherosclerosis is frequently neglected.[3]

Strong and McGill have opened this new line of investigation and have presented a first attempt to delineate the successive stages of the natural history of human coronary atherosclerosis. This natural history was correlated with age, sex, race, and environment, as well as with the presence of risk factors for coronary heart disease. The basic idea was formulated as follows: "to pursue a disease the investigator must know the natural history of the disease, when it starts, how it progresses, what stigmata it leaves and how it makes a patient sick or kills him".[4] A diagram presented 3 decades ago showed the concept of the authors on the natural history of atherosclerosis.[5] This diagram is probably one of the most frequently borrowed figures in the current literature. It includes an oversimplified model for the natural history of human atherosclerosis which describes the initiation of lesions in early childhood as fatty streaks and the evolution of fatty streaks to fibrous plaques and complicated lesions in middle and late adult life with the appearance of clinical manifestations.

The following succession of events has been suggested in the natural history of coronary atherosclerosis in New Orleans white males:[6] musculo-elastic thickenings appears in the first decade of life, fatty streaks in the second, fibrous plaques in the third, complicated lesions in the fourth, and clinical manifest disease in the fifth decade.

Based on observations made on human subjects at autopsy, the above-mentioned diagram indicates that human atherosclerosis starts invariably with fatty streaks in aorta, coronary arteries, cerebral arteries, and peripheral arteries. These apparently innocuous subendothelial foam cell accumulations progress to fibrous plaques and are responsible for the onset of coronary heart disease, cerebrovascular disease, aortic aneurysms, etc. In sum, fatty streaks are charged with the responsibility of the most important part of human arterial pathology.

The historical value of this attempt to construct the successive stages of the natural history of coronary atherosclerosis is unquestionable. We consider, on the other hand, the following:

1. The natural history of human coronary atherosclerosis cannot be covered by an oversimplified presentation, since it is not similar among the four major coronary arteries, between proximal and distal segments of a major coronary artery, or between a major coronary artery and its main branches.
2. Fatty streaks are not the unique early atherosclerotic lesions present in human coronary arteries or even the prevalent early lesion in some populations. In our material, for instance, fibromuscular plaques, gelatinous lesions, intimal necrotic areas, incorporated microthrombi, and intramural thrombi preceded the first fatty streaks visible on gross inspection and the prevalent early lesion was fibromuscular plaque.
3. The onset of six types of early atherosclerotic lesions cannot be confined to childhood or childhood and adolescence; they also appear during adulthood in various segments of the coronary arterial tree.
4. The role of fatty streak as the unique or even the main precursor of advanced coronary atherosclerotic lesions was not convincingly demonstrated in any published paper.
5. Fatty streaks cannot be considered atherosclerotic lesions in a similar sense as the term fibrous plaque; all plaques are lesions, whereas some fatty streaks may represent a physiological response of the reticulo-endothelial system to lipid overload.
6. The view that fatty streaks represent the unique arterial wall change that may lead to coronary heart disease, cerebrovascular disease, peripheral arteriopathies, and aortic aneurysms requires an adequate demonstration.

7. The complicated plaques are not a compulsory step between fibrous plaques and clinical manifestations induced by coronary atherosclerosis, both myocardial infarction and sudden cardiac death occurring in many subjects in the absence of complicated plaques.

Starting from the diagram of the natural history of atherosclerosis mentioned earlier, many investigators have taken preconceived ideas as well-established facts and in some works a similar, strange assumption exists: atherosclerosis begins during childhood with aortic fatty streaks which give rise in adults to coronary heart disease and cerebrovascular disease. Both coronary heart disease and cerebrovascular disease have nothing to do with aortic fatty streaks of children. Even if we accept, theoretically, that fatty streaks may give rise to fibrous plaques, the first fatty streaks appear in the coronary arteries during adolescence and in the cerebral arteries during early adulthood.

Investigations carried out in our laboratory showed that from 100 mature adults with coronary atherosclerotic plaques, only 4 would have these plaques from childhood, and only 12 from adolescence. This means that *in 84 out of 100 cases, plaques developed during adulthood.*

In our material, the first coronary atherosclerotic lesions appeared as fibromuscular plaques, gelatinous lesions, intimal necrotic areas, incorporated microthrombi, and intramural thrombi and not only as fatty streaks. On light microscopic examinations, fibromuscular plaques, gelatinous lesions, and incorporated microthrombi preceded by 5 to 8 years the first fatty streaks visible on gross inspection.

The fibromuscular plaque developed in our unselected Bucharest population sample as the most important early atherosclerotic lesion and as the main source of advanced fibronecrotic and fibrohyaline plaques. Its onset was recorded in the proximal segment of the left anterior descending artery in children aged 6 to 10 years in the left main coronary artery in adults aged 31 to 35 years, and in the atrioventricular node artery in adults aged 41 to 45 years. An interval of about 2 decades was detected between the first fibromuscular plaques in the proximal and the distal segments of the left circumflex artery, whereas in the right coronary artery fibromuscular plaques developed in the same period of time (adolescence) in the proximal and intermediate segments. We can offer many examples to demonstrate that *in the coronary arterial bed each vessel has its own natural history of atherosclerotic lesions.* Consequently, data recorded from the left main coronary artery cannot be superposed upon those recorded from the left anterior descending artery; the right coronary artery also has a particular natural history which is not superposable to that of the left circumflex artery. A particular natural history of atherosclerotic lesions exists in the posterior descending, first diagonal, first septal, left marginal and right marginal vessels, as well as in the vessels supplying the conduction system. Similar observations were made in our laboratory as concerns the intracranial arteries: the natural history of atherosclerosis of the vertebral artery is not superposable to that of the basilar artery, or that of the basilar artery to that of the anterior cerebral artery. Moreover, in the coronary and intracranial arteries the natural course of atherosclerotic involvement appeared different at branch sites from unbranched regions.[7-9]

These various patterns of the natural history are in agreement with the variety of etiologic agents and pathogenetic mechanisms involved in atherogenesis: agents that produce focal proliferations of smooth muscle cells giving rise to fibromuscular plaques; agents that produce intimal insudation, leading to the appearance of gelatinous lesions and of intimal necrotic areas; agents that produce intracellular lipid accumulations occurring as fatty dots and streaks; and agents that produce abnormal fibrinogen and fibrin accumulation and platelet aggregation, leading to incorporated microthrombi and intramural thrombi.

The fact that we observed that the natural history of coronary and cerebral atherosclerosis may begin the cycle of evolution in different arteries of the same vascular bed and in different

segments of the same artery during childhood, adolescence, and adulthood, suggests that atherogenic agents are present and active from childhood to adulthood. Consequently, sudden cardiac death may result from an obstructive atherosclerotic plaque developing in the right coronary artery starting from adolescence, but may also result from an obstructive atherosclerotic plaque developing in the atrioventricular node artery in subjects more than 40 years old. The end stages of these two lesions might be apparently similar, but the natural history is different not only as concerns the period of onset of lesions, but also their topography and pattern of progression. This example clearly demonstrates that all tendencies to oversimplification are unrealistic.

Whereas the responses of the coronary artery wall to atherogenic agents appear as at least six types of early atherosclerotic lesions, in a more advanced stage of the natural history these structural differences progressively disappear. As in many inflammatory and repair processes, the progression of lesions produced by various etiologic agents and pathogenetic mechanisms seems to be toward a common pathway which inevitably leads in the human coronary arteries to an advanced fibronecrotic or fibrohyaline plaque, with or without lipid deposits.

B. Coronary Atherosclerosis and Coronary Heart Diseases

The relationships between coronary atherosclerosis and coronary heart disease cover a wide area, with exponentially growing interest from a variety of different disciplines, ranging from cardiology, pediatrics, gerontology, pathology, physiology, biochemistry, biophysics, angiography, and pharmacology to cell biology, genetics, epidemiology, and experimental studies. In spite of this multidisciplinary approach many important problems still remain unsolved.

One of the major difficulties to relate coronary atherosclerosis to coronary heart disease and vice versa resides in the fact that coronary atherosclerosis itself has no proper signs and symptoms and can be revealed only by angiographic or pathologic investigations. On the other hand, an asymptomatic coronary atherosclerosis may produce progressive reduction of the coronary artery lumen followed by an imbalance between myocardial demands and coronary supply. The lesion is located in the coronary artery, whereas the clinical manifestations are myocardial: various forms of angina pectoris, myocardial infarction, and severe arrhythmias followed by sudden cardiac death. *The natural history of coronary atherosclerosis requires investigation of a peculiar disease in which the stenotic lesions develop in the coronary wall and the clinical manifestions are myocardial.* It is also necessary to note that such lesions are not the unique precondition able to produce myocardial clinical manifestations. Similar signs and symptoms may occur following coronary vasospasm, intracoronary emboli of platelet aggregates, coronary steal, myocardial necrosis of nonischemic origin, etc. These seem to be rare events; in the great majority of cases the main cause for the occurrence of myocardial clinical manifestations is coronary atherosclerotic plaques which encroach upon the vascular lumen and significantly reduce blood flow. This leads inevitably to the conclusion that "the importance of atherosclerosis is in direct proportion to its disease-producing potential".[10]

In an international study of the geographic pathology of atherosclerosis,[11] the intimal surface covered with fatty streaks and raised lesions (noncomplicated and complicated plaques) was compared among selected populations with diverse environmental and genetic backgrounds. This important investigation, designated the International Atherosclerosis Project, provided a cross-sectional description of atherosclerotic involvement as seen on gross inspection, showing that: (1) advanced atherosclerotic lesions may be visualized 2 or 3 decades before myocardial clinical manifestations; (2) the extent and severity of fibrous plaques in autopsied persons can be related to the mortality from myocardial infarction and sudden cardiac death in the respective populations; and (3) the risk of myocardial clinical manifes-

tations increases considerably when a certain threshold of severity was reached by atherosclerotic lesions.

The results of many other studies revealed that the most significant factors relating coronary atherosclerosis to coronary heart disease seem to be

1. The severity of atherosclerotic involvement
2. The association of stenotic or occlusive plaques with an inadequate development of collateral vessels
3. The sudden occurrence of occlusive thrombi on preexisting stenotic plaques
4. Conduction defects
5. Generalized impairment of the left ventricular functions
6. Significant involvement of the left main coronary artery and of the vessels supplying the conduction system

The view of the severity of coronary atherosclerosis is the main determining factor in the morbidity and mortality from coronary heart disease is largely accepted. Experienced pathologists and angiographers are also aware of problems that occur at the opposite end of the spectrum: patients who die suddenly without severe atherosclerotic involvement of the coronary arteries, or patients with myocardial ischemia or even infarction without obstructive plaques in the vessel supplying the infarcted area. There are also individuals with severe and extensive coronary atherosclerosis and even with occlusive plaques without myocardial clinical manifestations. Also of note is the routine observation that coronary heart disease patients at the onset of the myocardial clinical manifestations show severe stenotic or occlusive plaques considered to preexist months or years without clinical symptoms. This leads to the conclusion that "coronary heart disease seems to be a more complex phenomenon than explained by morphologic findings".[12]

To produce myocardial clinical manifestations, coronary atherosclerotic lesions must first develop, then progress to advanced forms able to produce luminal narrowing which significantly reduce coronary blood flow. Likewise, to give rise to myocardial clinical manifestations, such lesions must be not bypassed by adequate collateral vessels.

The first stage of the natural history of coronary atherosclerosis was characterized in our material by the onset of six types of early lesions: fibromuscular plaques, gelatinous lesions, intimal necrotic areas, fatty streaks, intramural thrombi, incorporated microthrombi. *All these early lesions developed in the presence of very low, low, medium, high, and very high blood cholesterol levels in normo- and hypertensive individuals, in nonsmokers and heavy smokers,* etc. In an unselected Bucharest population sample with a blood cholesterol level in children, adolescents, and young adults ranging from 110 to 160 mg/dℓ,[174] we revealed 58% young adults with coronary atherosclerotic plaques, 40% young adults with gelatinous lesions and intimal necrotic areas, and 2% with incorporated microthrombi and intramural thrombi.[13] Some plaques encroached upon the vessel lumen up to 75% in undistended coronary arteries.[14] Many other studies showed that spontaneous atherosclerotic lesions develop in the presence and absence of risk factors for coronary heart disease and particularly in subjects with low blood cholesterol levels, lacking other lipid metabolism abnormalities. On the other hand, these observations disagree with the view that the first stage of the natural history of atherosclerosis takes place in the blood and is preceded by hypercholesterolemia. Experimental studies show that whenever the level of blood cholesterol is elevated by dietary inclusion of excess cholesterol, sooner or later this is followed by the development of intraarterial lipid accumulations, occurring as fatty streak-like lesions. The fact that hypercholesterolemia is necessary to produce fatty streak-like lesions in experimental animals does not mean that it is also a prerequisite for the development of early atherosclerotic lesions in human coronary arteries. Too many investigators have accepted the validity of

experimental data, overlooking the fact that we are still unable to judge when the presence of a coronary fatty streak is good, bad, or indifferent.

There is a need to reemphasize that the onset of early lesions in the coronary arterial tree can be detected during childhood, adolescence, young, and late adulthood. Fibromuscular plaques develop during childhood in the proximal segment of the left anterior descending artery; during adolescence in the intermediate segment of the right coronary artery and the proximal segment of the left circumflex artery; during early adulthood in the proximal segment of the posterior descending and first diagonal arteries; and in mature adults in the first septal, second diagonal, left and right marginals, intermediate segment of the left anterior descending and circumflex arteries, and the vessels supplying the conduction system. Each segment of the coronary arterial tree has its own period of onset of early atherosclerotic lesions, strongly related to the branching anatomical pattern.[8,9,15,16]

The second stage of the natural history of coronary atherosclerosis includes the period in which the early lesions progress toward advanced lesions. In each segment of the coronary arterial tree, this progression acquires its particular feature[9] and takes place in its own period of time.[17] On selected topographic sites of the coronary arterial tree placed in sequence according to age, sex, and anatomical branching pattern, we found that (1) in subjects with a normal lifestyle who died in traffic accidents, the number of all early atherosclerotic lesions increased linearly and in parallel from one 5-year age group to the next, nonsignificant differences in the rate of progression being detected among lesions over a period of 25 years; (2) during this period of 25 years, the number of atherosclerotic plaques increased 4.7 times, gelatinous lesions and intimal necrotic areas increased 4.6 times, incorporated microthrombi and intramural thrombi increased 4.3 times, and fatty streaks increased 3.9 times.[17]

In cases submitted to the influence of the major risk factors for coronary heart disease (particularly heavy smokers), this age-related rate of occurrence and progression of early lesions toward advanced forms appears accelerated. The major risk factors for coronary heart disease produce not only rapid cycles of evolution from early lesions to lesions of possible clinical significance, but also aggravate the fate of preexisting plaques due to successive incorporation of microthrombi in the luminal half of the preexisting plaque, massive accumulation of extracellular lipid deposits in the necrotic center of the plaque, and augmentation in plaque size following edema, insudation, and development of a thick fibrous cap.

The onset of these advanced lesions characterizes the second period of the natural history of coronary atherosclerosis which persists as long as these advanced plaques remain asymptomatic. In our material and with the methods used, particularly the method of successive observations of similar topographic sites placed in sequence according to age, sex, and anatomical branching pattern, we revealed that *64% of advanced lesions have as a starting point a fibromuscular plaque, 24% gelatinous lesions and intimal necrotic areas, 10% incorporated microthrombi and intramural thrombi, and 2% fatty streaks.* If only the lesions present at branch sites are investigated, then only fibromuscular plaques appear as a early lesion. In unbranched segments the progression from early to advanced lesions is more complex, involving gelatinous lesions, intimal necrotic areas, and fatty streaks.[9]

The great majority of adults and elderly people who die of noncardiac causes have this second stage of the natural history of coronary atherosclerosis on post-mortem examination. A certain proportion may exhibit silent myocardial ischemia, documented by ECG abnormalities and thallium myocardial perfusion defects.[18]

The prevalence of asymptomatic individuals with silent myocardial ischemia in a given population is considered to be very important, but the diagnosis is in most cases fortuitous. Once thought to be an unusual phenomenon, confined largely to anecdotal reports of silent myocardial infarction, it is now recognized to be a common occurrence in middle-aged and elderly people. In certain ambulatory ECG studies, around 75% of ischemia cases were

clinically silent.[19,20] The per year mortality of such persons seems to be one half that of symptomatic patients.[21] Cardiologists are becoming increasingly interested in these apparently healthy persons who, upon close investigation, have signs of silent myocardial ischemia.[22]

Finally, the third or end stage of the natural history of coronary atherosclerosis makes its appearance with the first myocardial clinical manifestations: angina pectoris, myocardial infarction, and severe arrythmias followed by sudden cardiac death.

In some individuals, the passage from the asymptomatic to symptomatic stage of the natural history of coronary atherosclerosis is very rapid and may occur during childhood or adolescence. These are patients with Hurler's syndrome or with familial hypercholesterolemia, or with peculiar anatomical branching patterns of the coronary arteries. In addition, a more rapid transition from asymptomatic to symptomatic stages can be recorded in males than in females of a similar age, in white than in black races, in populations of industrialized countries as compared to underdeveloped ones, in heavy smokers as compared to nonsmokers, in hypertensive patients as compared to normotensive individuals, in diabetics as compared to nondiabetics, etc. There is a different type of lesion progression in the left main coronary artery as compared with the proximal segment of the left anterior descending artery; in the anterior descending as compared to the posterior descending artery; in the left circumflex as compared to the right coronary artery, etc. Each vascular segment of the coronary arterial tree has its own period and rate of progression of lesions and its own period of involvement of these lesions in myocardial clinical manifestations. This is the reason we were unable to present in this book schemas or diagrams which are valid for all the vessels included in the coronary arterial bed. For the same reason we have hesitated to reproduce schemas and diagrams existing in the available literature, since all attempts to gain more clarity by means of oversimplification may lead to unrealistic views.

The third or end stage of the natural history of coronary atherosclerosis is largely analyzed in Chapter 5 — some salient points are emphasized here. These points refer to the excessive morbidity and mortality among persons with unstable angina pectoris, or a previous myocardial infarction and to the rapid evolution of unstable angina toward myocardial infarction. In spite of this darker prognosis, the severity of coronary atherosclerotic lesions often appears similar in patients with stable and unstable angina. Also of note is the observation that sudden cardiac death may occur at many points in the panorama of the varied clinical story of the natural history of coronary atherosclerosis. There are persons who die from sudden cardiac death as the first myocardial clinical manifestation; others die suddenly from a second or third myocardial event. Individuals with an apparent similar severity and extension of coronary atherosclerosis are victims of sudden cardiac death at 40, 60, or 80 years of life.

The classical assumption which continues to be encountered in many books, reviews, schemas, and diagrams, that sudden cardiac death may be regarded as a myocardial infarction before necrosis has had time to become enzymatically or histopathologically manifest, appears as a debatable view. A large number of patients who have been successfully resuscitated from that would otherwise have been a lethal ventricular fibrillation, in the resuscitation period only one out of five of the survivors went on to develop myocardial infarction.[23,24]

When all the sudden cardiac deaths are indiscriminately lumped together, regardless of the period of occurrence in the course of the natural history of coronary atherosclerosis, it becomes difficult to determine which association of the changes has a pathogenetic significance for the early events leading to death. Moreover, the extension and severity of atherosclerotic involvement in the coronary arteries of individuals dying suddenly differs in no meaningful way from that observed in patients with angina pectoris or myocardial infarction, or in certain asymptomatic cases who die in traffic accidents.

There is good evidence to suggest that the contribution of three spheres of risk could be involved to produce sudden cardiac death: coronary atherosclerosis usually in the stage of stenotic or occlusive plaques, myocardial lesions able to induce severe arrhythmias; and

major or minor anomalies of the coronary arteries, the myocardium, and the conduction system able to induce myocardial ischemia associated with myocardial electrical instability. These three spheres represent a simplified view of a very complex problem. They cannot explain in each case why some die as mature adults and others as elderly people, why some die suddenly and others experience cardiac arrest and survival, and many others current pressing problems in cardiology. Only by recognizing the multifactorial nature of coronary atherosclerosis can we hope to develop an appropriate strategy to deal with as many of these problems as possible.

At present it is safe to say that

1. Coronary atherosclerosis is the main couse of myocardial clinical manifestations occurring as coronary heart disease, but these clinical manifestations may appear in the absence of severe atherosclerotic involvement of the coronary arteries
2. No single mechanism has been clearly implicated in atherogenesis or in the progression of atherosclerotic lesions
3. Progression from early to advanced coronary atherosclerotic lesions is not similar to progression to clinical disease
4. The risk factors for coronary heart disease are not necessarily the same as those for coronary atherosclerosis, nor do they have the same relevance to prevention and treatment
5. The control of coronary heart disease is not superposable with the control of coronary atherogenesis or with the control of progression of coronary atherosclerotic lesions
6. Whereas the agents which produce atherosclerotic lesions are largely unknown, we are tempted to believe that coronary heart disease usually develops when thrombosis is associated with preexisting stenotic plaques
7. Thrombi may form, disintegrate, produce peripheral emboli, and form again, sometimes leading to total occlusion of a major coronary artery and/or of a main branch vessel and this passage from a stabilized to a dynamic-aggressive lesion is closely related to the passage from asymptomatic to symptomatic myocardial ischemia

C. Problems Related to Early Prevention

The state of the art in this field, as in many other fields of atherosclerosis research, is reflected in different opinions published in the same year: "Should pediatricians be concerned about children's cholesterol levels? In the light of current knowledge, the answer in regard to most children must be a resounding no."[25] "Is atherosclerosis a pediatric problem? We believe that this question should be answered with a resounding yes."[26]

A recent investigation of the Bogalusa Heart Study[27] shows that in subjects with a mean age of 18 years at death, the coronary arteries were involved (percentage surface involvement): between 0 and 6.2% with fatty streaks and between 0 and 1.4% with fibrous plaques. In the same investigation, it is assumed that "the progression of fatty streaks to fibrous plaques is uncertain".[27]

In another recent investigation of the Bogalusa Heart Study,[28] the following is presented as well-established fact: "The atherosclerotic process begins early in life. Vascular changes progress from fatty streaks to fibrous plaques during young adulthood."[28] Many cardiologists, pediatricians, epidemiologists, and investigators in the field of atherosclerosis research believe that early intervention is critical if the natural history of coronary atherosclerosis is to be modified in an attempt to prevent myocardial infarction and sudden cardiac death. Therefore, the study of coronary atherosclerosis in children appears, theoretically at least, of paramount importance, since it might indicate:

1. The presence of atherosclerotic lesions and their prevalence in neonates, infants, children, and juveniles

2. The type of early lesion that prevails related to age, sex, race, environment, geographic pathology, etc.
3. The period of life in which these lesions begin to progress more rapidly in high-risk populations and individuals
4. The relationship between the severity of children's coronary atherosclerosis and the severity of lesions in adults in various geographic areas

In addition to data offered by post-mortem examination, many attempts are made by epidemiologists and clinical physicians to detect susceptible children or children considered at high risk. This is usually associated with an annual inventory of the family and the recommendation of preventive measures, such as dietary nutritional counseling, discouragement of smoking, control of blood pressure level, etc.

A meticulous light microscopic investigation of the coronary arteries of an unselected Bucharest population sample allowed us to reveal that 2% of children 6 to 10 years old showed fibromuscular plaques and an additional 2% exhibited gelatinous lesions and intimal necrotic areas.[13,29]

In other studies, an unexpected prevalence of microthrombi was revealed in children aged 1 to 4 years, more than 22% of these children showing this possible precursor of fibrohyaline plaques.[30,31]

Many pathologists could detect in the coronary arteries of newborn babies significant stenotic lesions.[32-40] In an autopsy series of 87 children under 1 week of age, coronary narrowings were found in over $^1/_5$ of the cases.[37] These coronary lesions present in newborn babies were related to associated infections and various injuries.[30,41] In particular, edema and insudation were frequently seen in the coronary arteries of infants — for instance, in 55 of the 134 cases dying in the first month of life.[42] In our material, infection was also related to the presence of very thick intimas in vessels of the coronary arterial tree with a diameter less than 1 mm, such as those supplying the conduction system.[43] Thickenings which encroached upon 50% of the coronary artery lumen were also recorded in the coronary arteries of Finnish children.[44]

The more frequent presence of a very thick coronary intima in boys as compared to girls, and in children belonging to an ethnic group with a high prevalence of coronary heart disease as compared to children from a group with a low prevalence was repeatedly mentioned.[15,32,33,38,40] We were also able to demonstrate that the susceptibility to atherosclerotic involvement can be related to the anatomical branching pattern of the coronary arteries.[15,16,43]

Routine examination of the coronary arteries of children in the post-mortem room revealed the unexpected frequency and severity with which these vessels could be altered. The available literature indicates that the most extensive and obstructive lesions can be found in patients with Hurler's syndrome, familial hypercholesterolemia, and Kawasaki disease.

The severity of plaque development and luminal narrowing in patients with Hurler's syndrome has been particularly emphasized, since such changes are produced by a mucopolysaccharidosis.[45,46] Kawasaki disease develops in infants and children with high fever, swelling of the lymph nodes, and characteristic mucocutaneous involvement. The coronary arteries exhibit severe atherosclerotic plaques, usually lipid-free, which may lead to myocardial infarction and sudden cardiac death during adolescence or young adulthood. These plaques also induce the development of many collateral vessels.[47,48] Data related to coronary artery changes in familial hypercholesterolemia were presented in the preceding chapter.

Many other results exist in the available literature which demonstrate that not all newborn babies, infants, children, and juveniles have normal coronary arteries similar to those of laboratory animals. Moreover, the coronary arteries of newborn babies, infants, children, and juveniles are submitted to particular processes of growth and remodeling which are sometimes difficult to delineate from pathologic changes. We were unable to find in the

available literature a comprehensive study on these age-related and pathologic changes and certain attempts in this respect acquire an inestimable value.[49]

Kannel is convinced that "lipids seem central to the atherosclerotic process beginning in childhood" and that is possible to present "pediatric aspects of lipid-induced atherogenesis".[50] This view might be supported by the lipid-laden cells detected in all children[51] and by the progressive age-related increased of esterified cholesterol in the apparently normal coronary arteries of children.[52] A significant positive correlation with age was recorded for both cholesterol ester fatty acid linoleate and arachidonate, suggesting as the main source of lipids plasma low density lipoprotein (LDL) particles. As judged by the observed gradual increase with age in arterial cholesterol esters and in its fatty acid composition, there is a continuous increase during childhood in LDL-derived esterified cholesterol in the subendothelial space, this augmentation being associated with changes in the amount of various types of glycosaminoglycans.[53] An important intra- and extracellular subendothelial accumulation of lipid was recorded in certain studies in children before[54] or after[55] the age of 10 years. The data offered by the Multicenter Study of Atherosclerosis Precursors in Finnish children and by other studies are also in agreement with the view that with age, the fatty acid composition of the coronary artery wall of children approaches that of blood LDL-particles.

A rise in serum total cholesterol from 65 mg/dℓ at birth, with 43% cholesterol in the high-density lipoprotein (HDL) particles, to 200 mg/dℓ at 30 to 35 years of age, with less than 30% cholesterol in the HDL particles is considered by certain investigators to be an atherogenic change. This deterioration of the LDL:HDL ratio in transition from childhood to adulthood is regarded as a preventable change through hygienic means in childhood. This prevention seems to be necessary in the light of the view that the dramatic rise in the LDL:HDL ratio "may be related to the early development of atherosclerosis".[28] In the Bogalusa Heart Study, a longitudinal increase of 13 mg/dℓ in LDL-cholesterol coupled with a simultaneous 11 mg/dℓ drop in HDL-cholesterol was associated with the development of early fibrous plaques. Likewise this dramatic rise was related to the higher risk for coronary heart disease death of young white men.[28]

Investigations carried out in the Bogalusa Heart Study also include important attempts to relate pathologic changes occurring in the coronary arteries of children and adolescents with changes in lipid metabolism.[27] The presence of coronary fatty streaks appeared correlated only with VLDL-cholesterol (VLDL = very low-density lipoprotein) and obesity was related neither to fatty streaks nor to raised lesions.

A noteworthy observation is that Venezuelan and North American schoolchildren have relatively similar total and LDL-cholesterol. On the other hand, Venezuelan children have considerably higher levels of triglycerides and lower levels of HDL-cholesterol than North American children.[56] These lipoprotein pattern differences are associated with a greater risk for coronary heart disease of North American adults.

Certain investigators think that to prevent coronary heart disease in adults it is necessary to maintain cholesterol levels of 120 to 160 mg/dℓ present in children.[57] Attempts to influence this level by means of diet, particularly the ratio of HDL-cholesterol vs. total cholesterol led to inconclusive results.[58] However, dietary n-3 polyunsaturated fatty acids, abundant in marine organisms seem to reduce the incidence of myocardial clinical manifestations,[59] but their role in atherogenesis is not clear.

It will be recalled that the atherogenic role of all types of lipids remains a subject of dispute as concerns human coronary arteries, whereas the role of lipids as a major risk factor for coronary heart disease is beyond dispute. Mean serum cholesterol levels of 100 to 150 mg/dℓ exist in most areas of the world where there is a small incidence of myocardial infarction. In populations with cholesterol value from 150 to 180 mg/dℓ, this incidence increases and according to worldwide evidence, high rates of myocardial clinical manifes-

tations are present in populations exceeding 200 mg/dℓ. These are populations with an excessive caloric intake caused by a high consumption of saturated fat and cholesterol, as well as a high consumption of sodium.

There is as yet insufficient evidence to establish whether atherosclerosis is in man a "pediatric nutritional problem", as suggested more than 25 years ago.[60] This view continues to be an essential part of epidemiological study and even the World Health Organization (WHO) founded a working group in 1974 on "precursors" of atherosclerosis in children, outlining some major problems in the field.[61] According to some results obtained in the Bogalusa Heart Study,[62-64] population intervention based on diet changes would seem justified. It is ironic that in Finnish children, the blood cholesterol levels are declining without active intervention. On the other hand, in certain countries the incidence of coronary heart disease continues to increase in spite of low cholesterol levels in children, adolescents, and young adults.

Obesity is also a risk factor suggested to be involved in coronary atherogenesis. It promotes most of the cardiovascular risk factors, including arterial hypertension, a poor LDL:HDL ratio and impaired glucose tolerance. In certain studies 10 to 30% of children were overweight and 10% had blood pressure values necessitating medical control and/or specific preventive action.[65] Fat children tend to become fat adults, particularly if they have fat parents. In normotensive people, the risk of arterial hypertension in subsequent years could be predicted from the degree of overweight and the amount of weight gain.[66] Nutritional intervention is important to reduce both obesity and arterial hypertension and many results show that weight reduction remains one of the most effective nonpharmacological means to reducing the atherogenic influence of arterial hypertension in children. A noteworthy observation is that the blood pressure can be increased or decreased in hypertensive or normotensive individuals by altering the fatty acid composition of the diet.[67]

The above-mentioned observations must be also related to an as yet-neglected risk factor in atherogenesis: the hepatic insufficiency of certain children. The uptake of chylomicron remnants by the liver (a receptor-mediated endocytic event in which apo E has a crucial role) can be significantly altered in hepatic insufficiency. The efficiency of this uptake is regarded by some investigators as a major determinant of the concentration of atherogenic lipoproteins.[68] In addition, the activity of hepatic LDL receptors determines to a substantial extent whether VLDL remnants, as well as LDL particles, accumulate in the blood and within the arterial wall. In all children with hepatic insufficiency it is tempting to presume that the role of hepatic LDL receptors in lipoprotein catabolism might be altered. Likewise, hepatic insufficiency might be associated with a decrease of the scavenger role attributed to reticulo-endothelial macrophages. The liver also produces anticoagulant proteins and hepatic insufficiency could act as an atherogenic mechanism if a prolonged state of hypercoagulable conditions exists. Clinical practice has demonstrated that some children are predisposed to thromboembolic phenomena and in such children microthrombi and intramural thrombi might be formed in the coronary arterial tree. As in the aorto-coronary saphenous vein graft, these microthrombi and intramural thrombi could represent the precursors of fibrous plaques with a rapid development and obstructive character.

The atherogenic role of smoking was not clearly demonstrated. However, this role is tacitly accepted and great effort must be expended by the physician to discourage smoking, especially in adolescents, before the habit becomes ingrained. Smoking leads to a stimulation of lipid metabolism (especially stimulated interconversion of triglyceride-rich lipoproteins), activates intravascular lipolysis, LDL formation, and decreases the HDL-cholesterol level. The suggested atherogenic role of smoking was also related to its capacity to increase the ability of blood to coagulate, particularly augmented platelet aggregability and adhesiveness. There are also many changes induced by smoking by means of abundant catecholamine release. The incubation of cultured human endothelial cells with cigarette smoke condensate

impaired prostacyclin (PGI_2) synthesis.[69] The by-products of smoking could produce endothelial and smooth muscle damage and alteration of various coagulation factor synthesis. This could be related to the tendency of smokers to be richer in microthrombi and intramural thrombi than nonsmokers of similar age and sex. Nicotine seems to be able to stimulate the synthesis and polymerization of the cytoskeletal protein in both endothelial and smooth muscle cells.[70] One might speculate that this kind of change in the cytoskeletal system could favor cell proliferation occurring as a fibromuscular plaque. The by-products of smoking may also act as premutagens and induce the onset of plaques following monoclonal cell proliferation.[71,72] Also, chronic exposure to carbon monoxide is related to an increase in vascular permeability, with the subsequent occurrence of subendothelial edema and gelatinous lesions. In our material, mucoid plaques (fibromuscular plaques with large areas of edema) were mainly detected in the coronary arteries of juveniles and adolescents who smoked 5 to 10 cigarettes each day.[73] On the other hand, there is evidence that smoking cessation improves myocardial performance, reduces the tendency to thrombogenesis, and may retard coronary atherogenesis and progression of preexisting lesions.

For more than 3 decades there has been speculation that adult coronary atherosclerosis has its origin in childhood. Certain investigators strongly believe that this is nothing more than a consequence of overindulgence in a diet too rich in saturated fat and cholesterol, sloth, and gluttony, leading to obesity, arterial hypertension, and cigarette smoking in the early teens.

While not denying the importance of epidemiologic studies during childhood and the presentation of children as miniature adults, we should like to adopt a cautious attitude when some investigators overemphasize this importance. All data in the available literature show that it is not possible to explain by means of quantitative variations of risk factors not only a large area of human pathology called coronary heart disease, but also the complex phenomenon of atherogenesis. In fact, all epidemiological studies are unable to explain two fundamental, current observations: (1) atherosclerotic lesions appear, develop, and progress in the absence of risk factors and (2) many victims of myocardial infarction or sudden cardiac death exhibit levels and patterns of risk factors compared to subjects without myocardial clinical manifestations.

From the totality of evidence developed in laboratory, clinical, and population studies, it is not possible to demonstrate that risk factors produce atherosclerotic lesions in human coronary arteries, but only aggravate preexisting lesions.

While the identification of the cardiovascular risk factors in children has enhanced knowledge and increased the necessity for primary prevention, this identification did not provide an adequate explanation for the agents and mechanisms involved in atherogenesis. Thus, for instance, the independent effect of family history may appear very important in some children who otherwise are at low risk.[74] This is regarded by some investigators as a proof that there are a host of unknown risk factors waiting to be identified. Other scientists consider that is not important to know how many risk factors can be found in children; what is very important is to demonstrate whether these risk factors are involved in the onset and progression of coronary atherosclerotic lesions. If this demonstration could be made, it must be associated with a primary prevention able to modify the natural history of coronary atherosclerosis starting from the first stage of this natural history.

Unfortunately, it is not yet possible to demonstrate that the interventions recommended in childhood will, in fact, lower the severity of atherosclerotic involvement in the coronary arteries of adults, but we tacitly accept that such interventions are necessary and desirable. There is a compelling need to protect children and adolescents from a so-called "atherogenic way of life" and it is tempting to presume that some factors which aggravate the natural history of coronary atherosclerosis arise, at least in part, from habits conditioned in childhood. In fact, we all agree that the high incidence of myocardial clinical manifestations induced

by coronary atherosclerosis in all industrialized countries does not permit indefinite temporizing, awaiting putative proofs. According to some optimistic views, the study of asymptomatic children forms the basis of optimum preventive cardiology and will undoubtedly have a greater impact on the decrease of cardiovascular diseases in the future.[62,75] We hasten to add that in spite of these optimistic views, the underlying mechanisms of atherogenesis in human coronary arteries are still a subject of debate and the reader is cautioned to critically assess data presented in the current literature.

II. ATHEROSCLEROTIC INVOLVEMENT OF THE CORONARY ARTERIES OF CHILDREN

A. Suggested Significance of Intimal Thickening

There is very little reliable information about the significance of very thick intimas present in human coronary arteries starting from birth. Opinions range from the view that they are normal developmental structures to the view that they are an integral part of atherosclerotic involvement. There is also evidence to suggest that the thickened intima of arteries may be regarded as a highly organized and effective repair and adaptive response to injuries.[76,77] Note the observation that the thickened intima may act as a site of clonal proliferation.[78]

A recent study in our laboratory revealed that in addition to its role in vascular remodeling and of susceptible sites for atherosclerotic involvement, intimal thickening itself may lead to changes similar to those produced by a fixed stenotic lesion.[43] Particularly in coronary vessels with a diameter of less than 2 mm, a very thick intima may significantly reduce the arterial lumen, in spite of an apparently normal microarchitecture. Coronary intimal thickening in Finnish children who died violently were found to reduce up to 50% luminal diameter.[44] In children many investigators have recorded electrocardiographic changes of myocardial ischemia without post-mortem presence of stenotic atherosclerotic plaques, but only of bulky intimal thickening. Clinical manifestations of angina pectoris were also attributed in children to bulky intimal thickening and in a study of infants up to 1 month old, unusually bulky thickened intimas were recorded in 22% of cases.[42]

Pathologists who carried out a light microscopic investigation of the thickened intima of the coronary arteries of stillborn babies, infants, and children can visualize the thickenings as a continuous range from least important to most important, from the apparently normal to the apparently obstructive intimal thickening. These significant differences from case to case and from one topographic area to another along the same vessel course were clearly revealed using the method of successive observation of similar topographic sites placed in sequence according to age, sex, and anatomical branching pattern (Tables 13 to 15). The results which also include separate data for males and females and the age-related dynamics of the male/female ratio show that a sex difference exists in the degree of intimal thickness in the left anterior descending and left circumflex arteries.

Intimal thickening which occurs in the coronary arteries of stillborn babies, infants, and children is considered by many investigators to be a physiological change that may result in a more adequate arterial wall remodeling, able to resist to the additional hemodynamic and mechanical stresses which appear after birth; however, when a bulky thickened intima is present the interpretation becomes very difficult. First, we must eliminate the possible existence of heart malformations; their incidence in certain population groups may rise up to 5% and decreases to 0.2% in subjects who live up to 15 years.[79] In the absence of heart malformations, the presence of a bulky thickened intima suggests the existence of a particular type of injury superimposed on a physiological growth and remodeling. This injury induced an abnormal migration of smooth muscle cells from media into the subendothelial area, followed by abundant new formation of ground substance and fibers.

Among the various injuries, the attention of certain scientists was mainly centered on

Table 13
SEX DIFFERENCES IN INTIMAL THICKNESS

Age groups (years)	Intimal thickness (μm)				
	Males		Females		
	Mean	Range	Mean	Range	M/F ratio
Left anterior descending artery (unbranched segment, 1 cm from point of origin)					
Neonates	61	0—112	35	0—73	1.71
1—5	78	12—165	49	0—104	1.59
6—10	96	46—188	64	0—143	1.50
11—15	138	68—203	98	16—186	1.40
Left circumflex artery (unbranched segment, 1 cm from point of origin)					
Neonates	40	0—76	26	0—42	1.53
1—5	54	0—133	38	0—66	1.42
6—10	62	0—175	48	0—75	1.29
11—15	108	20—199	76	16—103	1.42
Right coronary artery (unbranched segment, 2 cm from aortic ostium)					
Neonates	39	0—66	35	0—68	1.11
1—5	62	0—101	50	0—108	1.24
6—10	75	0—152	71	0—165	1.05
11—15	108	10—185	103	8—177	1.04

Table 14
SEX DIFFERENCES IN SEVERE CHANGES OF THE CORONARY INTERNAL ELASTIC MEMBRANE ASSOCIATED WITH INTIMAL THICKENING (% OF CASES)

Age groups (years)	Sex	Severe changes of the internal elastic membrane					
		Focal lysis		Fragmentation		Interruptions	
		PPC	M/F	PPC	M/F	PPC	M/F
Neonates	M	0		76		24	
	F	0		20	3.80	4	6.00
1—5	M	4		80		32	
	F	0		24	3.33	8	4.00
6—10	M	12		84		40	
	F	2	6.00	36	2.33	16	2.50
11—15	M	24		84		60	
	F	4	6.00	44	1.90	28	2.14

Abbreviations: M, males; F, females; PPC, percent of positive cases; M/F, male vs. female ratio.

arterial hypertension. Both transitory arterial hypertension[80] and hypoxia[81] are considered to be contributors to the development of a thickened intima as a result of catecholamine release; epinephrine alone appeared able to induce in adequate experimental models an important degree of intimal thickening. Similar pathogenetic mechanisms seem to operate when intimal thickenings develop following exposure to extremely stressful situations (neurogenic stresses). In line with this view, intimal thickening is rare in the coronary arteries in wild immature monkeys, but exhibits a considerable development after a short period of captivity.[82] It was

Table 15
PERCENT OF CASES WITH INTIMAL THICKENING IN SELECTED TOPOGRAPHIC SITES OF THE CORONARY ARTERIAL BED

Vessel studied	Age groups (years)		
	1—5	6—10	11—15
Right coronary artery at			
0.5 cm	0	0	0
2.0 cm	0	12 (0.61)	78 (0.68)
3.0 cm	0	16 (0.23)	77 (0.65)
4.0 cm	0	16 (0.93)	72 (0.56)
5.0 cm	0	18 (1.66)	75 (1.63)
Left main coronary artery at 0.5 cm	0	0	10 (0.66)
Anterior descending artery at			
2.0 cm	38 (0.83)	59 (1.03)	94 (1.96)
3.0 cm	22 (0.93)	46 (0.98)	81 (1.46)
4.0 cm	3 (0.32)	12 (0.63)	52 (0.75)
5.0 cm	0	0	19 (0.17)
Circumflex artery at			
1.0 cm	16 (0.75)	18 (0.78)	19 (0.98)
2.0 cm	17 (0.84)	21 (1.01)	28 (0.97)
Conus artery	0	0	0
Sinus node artery	0	0	5 (2.02)
Right marginal artery	0	0	2 (0.33)
Atrioventricular node artery	0	0	3 (2.35)
Posterior descending artery	0	9 (0.53)	14 (1.28)
First diagonal artery	0	0	8 (0.31)
First septal artery	0	0	5 (1.78)
Left marginal artery	0	0	0

Note: In parentheses, the highest value of the intima: media thickness ratio recorded.

also possible to produce coronary intimal thickening in repeatedly bred rats,[83] or after abrupt cessation of lactation,[84] or in rats maintained on vitamin E-deficient diet.[85]

The role of generalized infections in the onset and development of a coronary thickened intima was emphasized by many investigators.[39,42] In our material, approximately 1/3 of children 1 to 10 years old showed the development of a mucoid connective tissue with a honeycomb structure in the subendothelial zone and this was mainly seen in cases with generalized infections.[43] As has already been mentioned, these intimal thickenings associated with the presence of generalized infections mainly involved small coronary vessels and especially those supplying the conduction system.

All these types of intimal thickenings have little to do with the thickened intima produced in laboratory animals by feeding rabbits, rats, or monkeys with cholesterol-rich diets. Intimal thickening was also produced experimentally by various mechanical trauma (scarification, scraping of the arterial wall with angulated needles, insertion of a wire coil, distension with a balloon catheter introduced into the lumen, various types of external compressions), air embolism, injection of particulate suspensions, physical insults such as those obtained by freezing methods, chemical injuries, immune reactions, irradiations, injection of blood clots, etc. Whether these various types of injuries have any relevance to changes which appear in the coronary arteries of newborn babies, infants, and children is a question that cannot be answered with certainty.

The intensity of the local injury was also related to intimal thickening: a superficial trauma

may produce only transient intimal thickening, whereas strong injuries may produce intimal thickenings exhibiting a nonregressive character.[86] This response to injury may appear as an aberrant mesenchymal reaction, capable of providing a locus for the ulterior development of an atherosclerotic plaque.[87] In line with this concept, certain investigators have integrated some areas and some forms of intimal thickening in the nonlipid phase of atherosclerosis.[88-90] The response of the arterial wall to a hypothetic atherogenic stimulus might appear as diffuse intimal thickening if the agent acts over prolonged periods and in mild doses and as patchy atherosclerotic lesions if the agent acts in heavier doses.[91] It is interesting to note the absence of spontaneous atherosclerosis in animals lacking arterial intimal thickening.

There is convincing evidence to date that as long as the intimal layer is composed of only musculo-elastic elements, little lipid appears in this thickened intima, but when intima contains an additional superposed elastic hyperplastic and/or ground substance-rich sublayer, lipid accumulations are frequently detected. From a pathologic viewpoint, it is clear that lipid accumulations does not appear to be associated with musculo-elastic thickenings and does not cause this musculo-elastic proliferation; on the other hand, it seems closely associated with elastic hyperplastic and collagenous intimal thickening.[92] This can be also demonstrated in pigs, since only the elastic hyperplastic sublayer which develops later than the musculo-elastic sublayer is the site of lipid accumulations.[93] It is thus tempting to assume that intimal thickening develops in two stages: the first to appear is a musculo-elastic sublayer considered to represent a response to progressive hemodynamic changes occurring with increasing age; internal to this musculo-elastic sublayer a special type of intimal connective tissue sometimes occurs, which is regarded as a focal proliferative response to injuries of one sort or another. This sublayer may acquire a nodular character and could represent an integral part of coronary atherosclerotic involvement.[94] The view on the existence of an atherogenic intimal connective tissue is supported by observations carried out on vein grafts placed in arteries. The proliferation of cells in the subendothelial zone which gives rise to an intimal connective tissue starts within the first month after surgery and can lead to graft occlusion within the first year; this proliferation is of a nodular type and does not appear as a musculo-elastic sublayer.[95] In the aorto-coronary bypass grafts the thickened intima appears as a prerequisite for the development of atherosclerotic plaques. Concomitantly, it may act as a fixed stenotic lesion.

A particular type of intimal thickening was detected during our study on the natural history of coronary atherosclerosis in children with Hurler's syndrome and in children with familial hypercholesterolemia. Figure 68 shows that this intima was not only considerably thicker than the underlying media, but also acquired a nodular character. Histologically, it appeared similar to a fibromuscular plaque. Moreover, in a child with a chronic kidney disease and severe arterial hypertension we found that the thickened intima not only showed a light microscopic feature similar to a fibromuscular plaque, but also included areas rich in hyalinized tissue similar to the fibrous cap of fibronecrotic plaques (Figure 69). The tendency of these very thick intimas to reduce coronary artery lumen was often recorded in our material in both major coronary arteries and main branch vessels, particularly in children more than 3 years old.

A relationship was detected between the degree and extension of intimal thickenings on the one hand and genetically induced minor deviations from the common type of distribution of the coronary arteries on the other hand.[15,43] In similar topographic sites, intimal thickening developed 5 to 15 years earlier in subjects with than in subjects without atherogenic deviations from the common type of distribution of the coronary arteries. All children who showed a well-developed thickened intima in the left circumflex artery starting from the 1 to 5 year old age group exhibited a left circumflex artery more rapidly developed associated with a short and narrow right coronary artery which ended as the marginal branch. Other examples have been given in previous works,[15,43] and the results strongly suggest that *some intimal thickenings in the coronary arteries of children are branching-pattern dependent.*

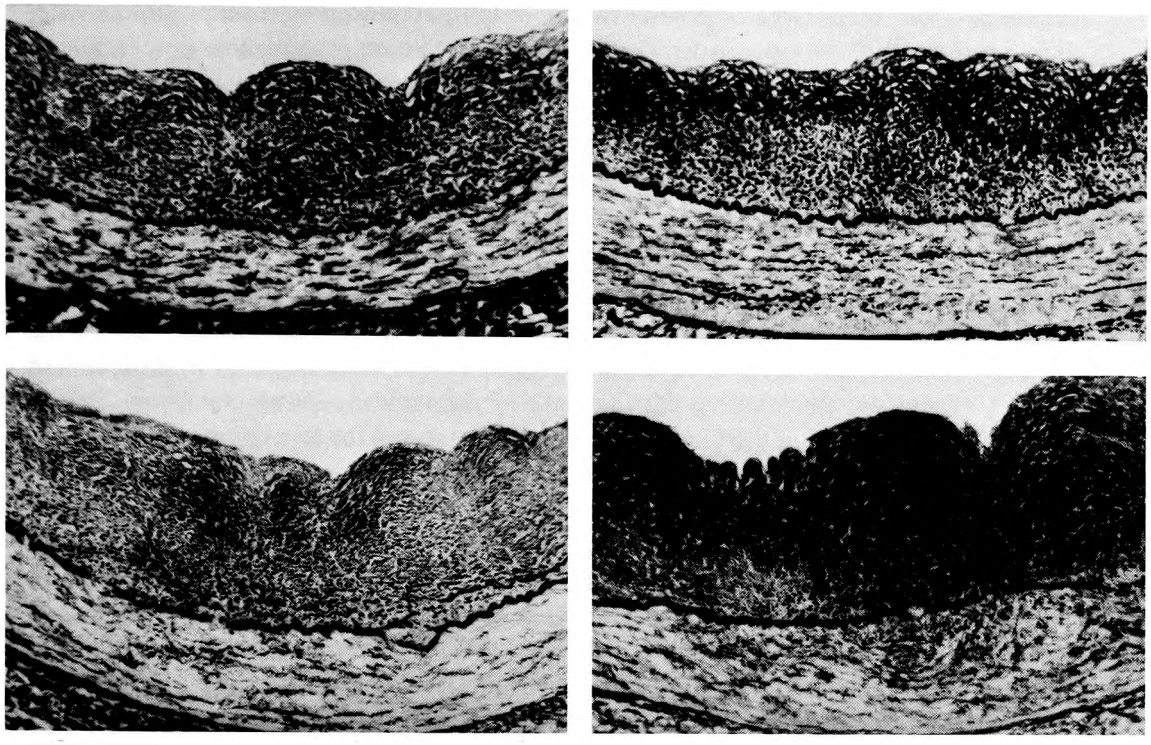


FIGURE 68. The proximal segment of the left anterior descending artery in four children 6 to 12 years old with Hurler syndrome (left) and familial hypercholesterolemia (right). The intima is not only considerably thicker than the underlying media, but has acquired a nodular character, its histological feature being similar to that of a fibromuscular plaque. (Resorcin fuchsin-alcian blue. Magnification × 120.)

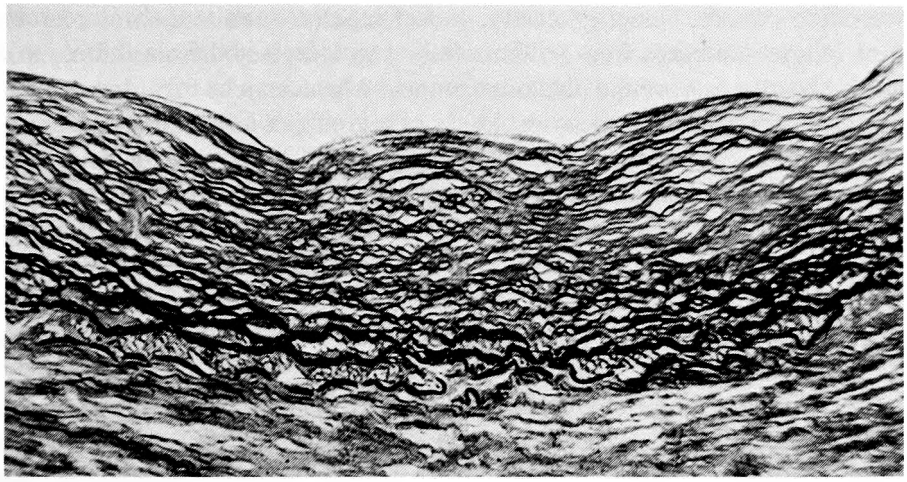


FIGURE 69. The proximal segment of the left anterior descending artery in an 11-year-old child with a chronic and severe kidney disease and arterial hypertension. The light microscopic feature of the thickened intima is similar to that of fibrohyaline plaques or to that of fibrous caps of fibronecrotic plaques. (Resorcin fuchsin-alcian blue. Magnification × 220.)

The role of genetic factors can also be correlated with differences in intimal thickness between male and female children revealed 4 decades ago.[32] We found not only that females have less intima than males of similar age, but also that the internal elastic membrane of the female coronary arteries of neonates, children, and juveniles exhibited a lesser tendency to fragment, interrupt, and show areas of elastolysis than did the male subjects.[96]

Additional proof on the role of genetic factors in intimal thickening resulted from studies on different population groups. In Israel, the children of ethnic groups more susceptible as adults to coronary heart disease developed a much thicker intima than the children of ethnic groups less susceptible as adults to myocardial clinical manifestations induced by coronary atherosclerosis.[38] In Finland, the coronary intimal layer showed a significant difference in thickness between eastern and western children. The association of infants having thick intima in the coronary arteries with a population group with a very high mortality from coronary heart disease was very demonstrative, and no other significant correlation was observed.[40] The difference found in the degree of intimal thickness between the coronary arteries of the eastern and western groups was attributed to a genetic susceptibility of the intimal layer to injuries. On the other hand, similar changes were detected in African and European neonates as concerns the development of the musculo-elastic sublayer. This is additional proof that splitting the internal elastic membrane and the formation of a thickened intima occurring only as a musculo-elastic layer play little part in determining the natural history of coronary atherosclerosis.[97] It is interesting to note that similar changes leading to the onset of a musculo-elastic intimal thickening were also observed in Yemenite fetuses and infants up to 1 year old, in non-Yemenite infants, and in infants from North America and the industrialized countries of Europe.[98] These changes are considered physiological in nature and related to age-dependent influences of hemodynamic stresses. Based on the succession of these changes, the following stages were suggested to occur within the first 12 months of life: (1) a reactive stage, (2) a proliferative stage, and (3) an adaptive stage.[99,100] The reactive stage includes severe changes of the internal elastic membrane, the proliferative stage an abundant formation of ground substance, fibers, and cells, and the adaptive stage the formation of the musculo-elastic sublayer.

The interpretation of the results of studies dealing with coronary intimal thickening is very difficult since we do not have a clear definition of the term "normal intima". In some reports, the term normal is used as equivalent of usual or expected thickness, but there are significant differences in the most homogeneous subgroups related to age, sex, race, anatomical branching pattern, vessel geometry, vessel segment, and vessel microarchitecture. Is the rise of intimal thickness from stillborn babies to infants and from infants to children always or only sometimes a normal phenomenon, and when can it be regarded as a pathologic one? What deviations above the mean thickness of a group of infants or children should be regarded as pathologic? We have mentioned in a recent work[43] that while a serum cholesterol level three times greater than the largely accepted normal level is considered an alarming pathological change, an intimal layer three times thicker than the underlying media is presented as a normal developmental structure. Are the normal values recorded in primitive societies or in individuals not susceptible to atherosclerotic involvement comparable to those recorded in industrialized countries and in population groups prone to present myocardial clinical manifestations in adult life?

Since intimal thickening in the children's coronary arteries appears as a continuous variable, without a proper understanding of the normal, exploring the abnormal is a very difficult task. Experienced pathologists are familiar with the fact that the left anterior descending artery is the vessel of the coronary arterial tree with the most important degree of intimal thickening. They are also familiar with the fact that an excessive degree of intimal thickening usually appears in the premural portion of the proximal segment, whereas in its intramural portion, when myocardial tunnels or bridges exist, intimal thickening is absent or negligible. This clearly demonstrates that *a limitation of pulsatile flow distension by the ensheathing heart muscle in some way prevents intimal thickening*. Can we accept a normal value of intimal thickening for the premural portion and a totally different normal value for the intramural portion of the same artery? Additionally, if we accept these two different values, what is the significance of the mean degree of intimal thickness of the whole vessel reported by us and others?

In summary, we can assume that in the coronary arteries of children, intimal thickening occurring only as a reduplication of the internal elastic membrane and formation of a musculoelastic sublayer seems not to be associated with atherosclerotic involvement. On the other hand, intimal thickening including several sublayers and especially subendothelial elastic hyperplastic and ground-substance-rich sublayers, appears susceptible to the onset of atherosclerotic lesions. They may develop as *nodular proliferations* (occurring as fibromuscular plaques), as *focal areas of insudation* (occurring as gelatinous lesions and intimal necrotic areas), as *incorporated microthrombi* and *intramural thrombi* (which may give rise to fibrohyaline plaques), and finally as *intra-* and *extracellular lipid accumulations* (which may lead to the appearance of fatty streak-like lesions, to foam cell-rich plaques, and necrotic plaques rich in lipid deposits). The existence of multiple etiologic agents of human coronary atherosclerosis might be associated with the occurrence of many types of intimal thickenings susceptible to these agents or induced by these agents.

B. Atherosclerotic Plaques

The first atherosclerotic lesions detected on light microscopic examination of the coronary arterial tree of infants and children were, in our material, *the fibromuscular plaques which developed in places previously occupied by branch pads or cushions.*[29]

Intimal pads or cushions at sites of branching in the distributing arteries are considered analogous to intimal thickening, differing only in location and not in nature.[101] They are regarded as physiological or adaptive responses to the increase in blood pressure and vessel size and also as precursors of atherosclerotic plaques.[100-103]

The cusion-like thickenings probably represent specialized structures which cope with a high shear stress and/or longitudinal tension.[104] With growth, considerable remodeling occurs at bifurcations and branchings and results in the formation of pads or cushions which protrude into the lumen. The smooth muscle cells may exhibit a circular arrangement and even a microarchitecture of a sphincteric type.[105,106] The circular arrangement of the elastic and collagen fibers also represents a structural accomodation to the increased circumferential tension to which the branch mouths are presumably subjected.

The similarity between the topography of the adult localized fibrous plaques[35] and mucoid-fibromuscular plaques[87] or of raised lesions[107] and branch pads or cushions of the coronary arteries has repeatedly been emphasized. Likewise, the possible conversion of branch pads or cushions into atherosclerotic plaques in cerebral and other organ arteries in adults has been suggested. In rhesus monkeys fed an atherogenic diet, the most severe lesions of the coronary arteries developed in the large-shaped pads or cushions at the apex of the bifurcation of the left main coronary artery.[108]

Branch pads or cushions are normal anatomic structures in the sense that they are constantly present in the coronary arteries of fetuses, infants, and children, as well as in other organ arteries (see Figures 1 to 7). In our material, these anatomic structures occurred as a nidus for the onset of the first atherosclerotic lesions visible on light microscopic examination, the fibromuscular plaques. Likewise, branch pads or cushions of the coronary arteries act as a nidus for the progression of fibromuscular plaques toward advanced fibrohyaline and fibronecrotic plaques (see Figures 47 and 48).

Whereas fatty streaks could not be seen on gross inspection and light microscopic examination in children aged 6 to 10 years, the first fibromuscular plaques appeared in 2% of cases. These cases were male subjects with a minor deviation from the common type of distribution of the coronary arteries, characterized by a rapid development of the left anterior descending artery, associated with an underdeveloped posterior descending artery. These first atherosclerotic lesions were located at the point of origin of the left anterior descending artery (bifurcation area of the left main coronary artery) and in the proximal segment of the left anterior descending artery, at the point of origin of the first septal or diagonal vessels.

They encroached upon the lumen of the major coronary artery narrowing up to $^1/_3$ of the original lumen and appeared as homogeneous fibromuscular proliferations, exhibiting *a different microarchitecture than that of the preexistent branch pads.*

In children and juveniles aged 11 to 15 years, fibromuscular plaques were also detected only microscopically and occurred in 4% of cases. All of the plaque mass lies in the intima beneath the endothelium and entirely on the luminal side of the internal elastic membrane of the coronary arteries. These masses are sharply demarcated from the adjacent intima and their cell components appear to be smaller than cells of the adjacent intima and underlying media. The main extracellular material within these first fibromuscular plaques is collagen, whereas elastin intermingled with collagen are the major components of the adjacent thickened intima and of the media matrix. *The arrangement of cells in the plaque lacks the order found in the coronary media, but the most important and peculiar aspect is the presence of an excessive amount of cells, an apparently useless mass of cells.*

The center of the plaque is lipid-free, but its base and the adjacent internal elastic membrane are encrusted with lipids, stainable by both Sudan and OTAN methods. The intima adjacent to these fibromuscular plaques may include scattered monocyte-macrophage and few scattered lipid-laden cells.

Branch pads or cushions appear as heterogeneous structures, including a basal musculoelastic sublayer, an intermediate elastic hyperplastic sublayer, and a superficial or subendothelial ground substance-rich sublayer. Fibromuscular plaques occur as homogeneous structures, including a dense nodular agglomeration of smooth muscle cells and fine collagen fibers, dispersed in an abundant ground substance. This histologic difference would indicate that if a conversion of branch pads or cusions into fibromuscular plaques took place, it would require reorganization and homogenization of the branch pad or cushion microarchitecture. By placing, in an adequate sequence, according to age, sex, and anatomical branching pattern, the tissue sections obtained from branch pads located at similar topographic sites, several types of changes were revealed:[29]

1. Simple pad enlargement, sometimes with a considerable increase in its diameters, leading to the aspect of bulky intimal thickening which encroaches upon the vessel lumen
2. Interdigitation of neighboring pads, followed by their coalescence and the appearance of a diffuse thickened intima at the bifurcation area or branch site
3. Intrapad hyperplasia of elastic and collagen fibers with persistence of the preexisting heterogeneous microarchitecture
4. Intrapad edema, histolysis (elastolysis, collagenolysis, ground substance depletion, and degenerative cell changes), followed by reorganization and homogenization of the preexisting heterogeneous microarchitecture
5. Intrication of the above-mentioned degenerative and histolytic changes with reparative ones which occurred as nodular proliferations of smooth muscle cells, synthesis of ground substance rich in chondroitin sulfates, and fine collagen fibers

More than 14% of children and juveniles 11 to 15 years old showed in their coronary arteries branch pads or cushions in which the preexisting heterogeneous microarchitecture was progressively replaced by areas of histolysis, intermingled with nodular proliferations of smooth msucle cells, abundant new formation of fine collagen fibers, and accumulation of a chondroitin sulfate-rich ground substance. Some of the pads submitted to this reorganization preserved, in part, the tissue section their intial heterogeneous microarchitecture, whereas in other parts they appeared as developing fibromuscular plaques with their characteristic homogeneous microarchitecture. Particularly in the coronary arteries sectioned along their length in the branching region, what appeared to be a pad at one end of the

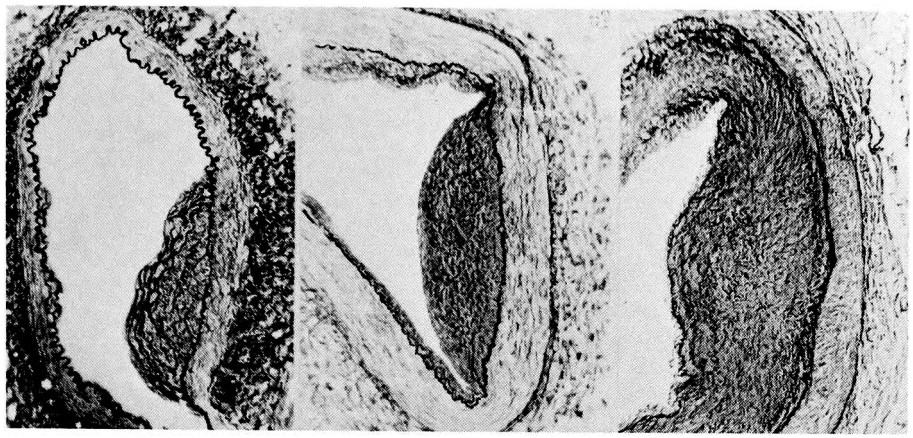


FIGURE 70. The proximal segment of the left anterior descending artery at the branching point of the first septal vessel. (Left) A typical branch pad or cushion in a newborn child; (middle) a transitional stage between a branch pad and a fibromuscular plaque in a 5-year-old child; (right) a typical fibromuscular plaque in a 9-year-old child. (From Velican, C. and Velican, D., *Acta Anat.*, 99, 377, 1977. With permission.)

eccentric thickened intima merged imperceptibly into a developing fibromuscular plaque at the other end.

The use of the method of successive observations of similar topographic sites placed in sequence according to age showed a progressive increase from newborn babies to infants and from infants to children and finally from children to juveniles in the number of pads with edema, histolysis, and structural reorganization and homogenization. On the other hand, only a very limited number of these pads acquired with age the light microscopic feature of an early atherosclerotic plaque (Figures 70 to 72).

During these suggested sequential steps occurring as very early stages in the natural history of human coronary atherosclerosis, we did not observe important lipid or fibrin accumulations, intimal hemorrhage, intimal vascularization, or pathological changes of the underlying media, adventitia, or the vasa vasorum. Also, we were unable to detect the accumulation of monocyte-macrophages or of leukocytes. On the other hand, we found that all branch pads submitted to edema and histolysis exhibited diminution of their ground substance metachromasia and basophilia and of collagen fiber fuchsinophilia. Depletion particularly involved the proteoglycans resistant to hyaluronidases and chondroitinases (heparan sulfates and other heparin-like substances). When histolysis became intense, it appeared associated with diminution in the histochemical reactivity of dermatan sulfate, whereas passage from the prevalence of histolysis to the prevalence of proliferation led to progressive augmentation in the histochemical reactivity of chondroitin sulfates. This prevalent histochemical reactivity of chondroitin sulfates was revealed in our material in all developing fibromuscular plaques of the coronary arteries of children. An additional prevalent change was the disintegration of all thick elastic fibers and laminae present in the branch pad or cushion, sometimes in an important number. Consequently, the intermediate stages between the heterogeneous branch pad microarchitecture rich in elastic fibers and the homogeneous fibromuscular plaque microarchitecture lacking elastic fibers exhibited the light microscopic features illustrated in Figure 73.

Since the number of branch pads or cushions which showed areas of histolysis was six to seven times greater than the number of branch pads or cushions which showed nodular proliferations of smooth muscle cells, it might be assumed that branch pad histolysis is a precondition for structural reorganization and occurrence of fibromuscular plaques. On the other hand, not all branch pads with areas of edema and histolysis will give rise to such

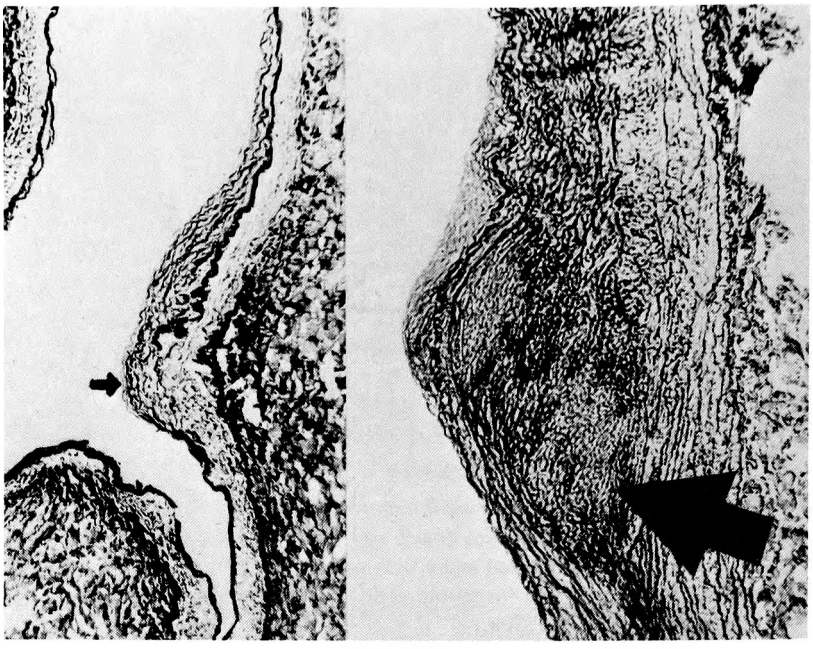


FIGURE 71. (Left) Small branch pad (small arrow) located near the branch mouth of the first diagonal branch of the left anterior descending artery of a neonate. (Right) A similar topographic site in a 12-year-old juvenile showing a very large pad with islands of histolysis (large arrow) as a result of elastolysis, collagenolysis, ground substance depletion, and degenerative cell changes. This is considered an intermediate step in the passage from the heterogeneous microarchitecture of branch pad to the homogeneous microarchitecture of fibromuscular plaque. (From Velican, C. and Velican, D., *Atherosclerosis*, 33, 201, 1979. With permission.)

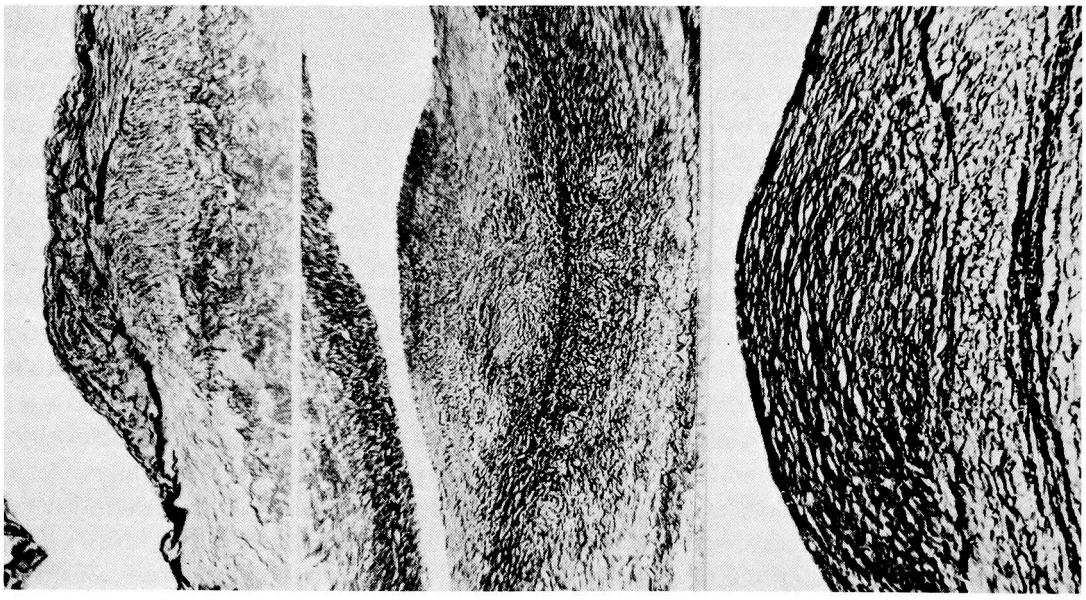


FIGURE 72. (Left) Bifurcation area of the left main coronary artery of a neonate with a branch pad located near the branch mouth of the left anterior descending artery. (Middle) Similar topographic site in a 6-year-old child showing diffuse histolysis as an intermediate stage in the passage from pad to fibromuscular plaque. (Right) Similar topographic site in a 15-year-old juvenile; a typical fibromuscular plaque is present. (From Velican, C. and Velican, D., *Atherosclerosis*, 33, 201, 1979. With permission.)

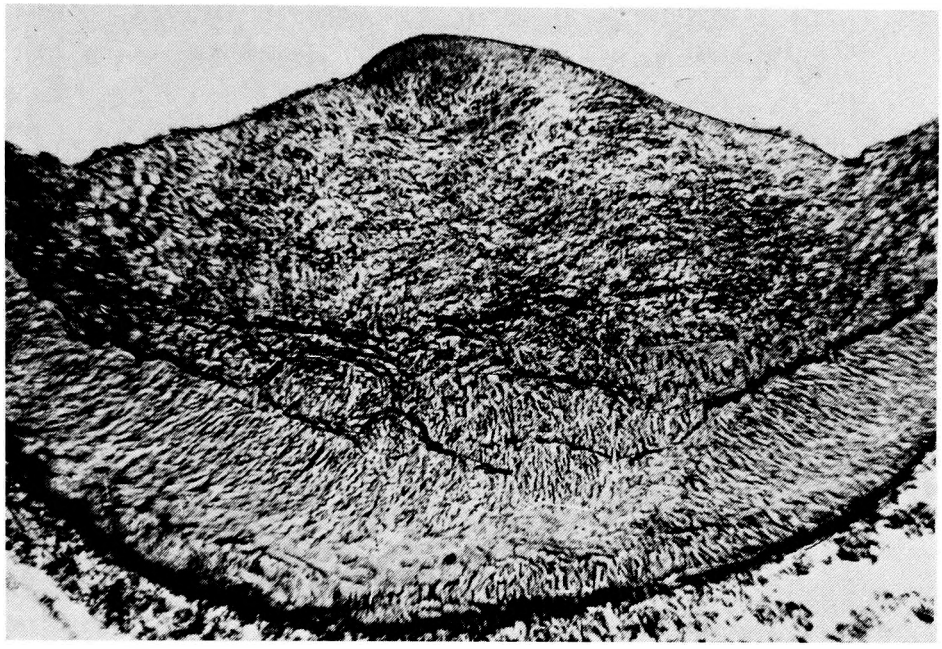


FIGURE 73. Intermediate stage between a branch pad or cushion and a fibromuscular plaque frequently detected in the coronary arteries of children and juveniles. Left circumflex artery of a 12-year-old juvenile at the branch site of the left marginal vessel. (Resorcin fuchsin-alcian blue. Magnification × 440.)

plaques; some remain as such, others are integrated within the diffuse thickened intima, and still others will accomplish this conversion in adolescents and young adults. One can also speculate that the rate of conversion could be accelerated by a particular pattern of hemodynamic stresses and by intervention of other atherogenic factors. Some observations suggest that when insudation prevails, the branch pad or cushion may be converted into a gelatinous lesion; when microthrombi encrustation and incorporation prevails, the branch pad or cushion may give rise to a fibrohyaline plaque. Finally, when necrosis prevails, the branch pad or cushion may include a necrotic area which progresses to a necrotic and then to a fibronecrotic plaque. These branch sites in which fibromuscular plaques develop are usually spared by fatty streaks even in young and mature adults. On light microscopic examination, the first fibromuscular plaques were detected 5 to 8 years earlier than the first fatty streaks. In a gross investigation on atherosclerosis of the coronary arteries in five towns,[109] the first fibrous plaques were detected as early as the first fatty streaks in the 10 to 14 years age group.

The above-mentioned observations were performed on apparently healthy subjects who died in traffic accidents. Our material also included newborn babies, infants, children, and juveniles hospitalized for various noncardiac diseases. In this selected sample we revealed in the coronary arteries of children not only fibromuscular plaques, but also more advanced atherosclerotic lesions. In agreement with other pathologists,[15,46] we recorded the most severe atherosclerotic plaques in children with Hurler's syndrome. In these patients with genetic absence of α-iduronidase and extensive and universal deposition of dermatan sulfate and heparan sulfate in various organs and tissues, the natural history of coronary atherosclerosis is dramatically accelerated. Narrowing by fibrohyaline plaques of the major coronary arteries revealed at necropsy is both severe and diffuse and is associated with a diffuse thickening of all cardiac valves and of ventricular endocardium. An analytical study revealed that at least one of the four major coronary arteries were narrowed 76 to 100% and in some of

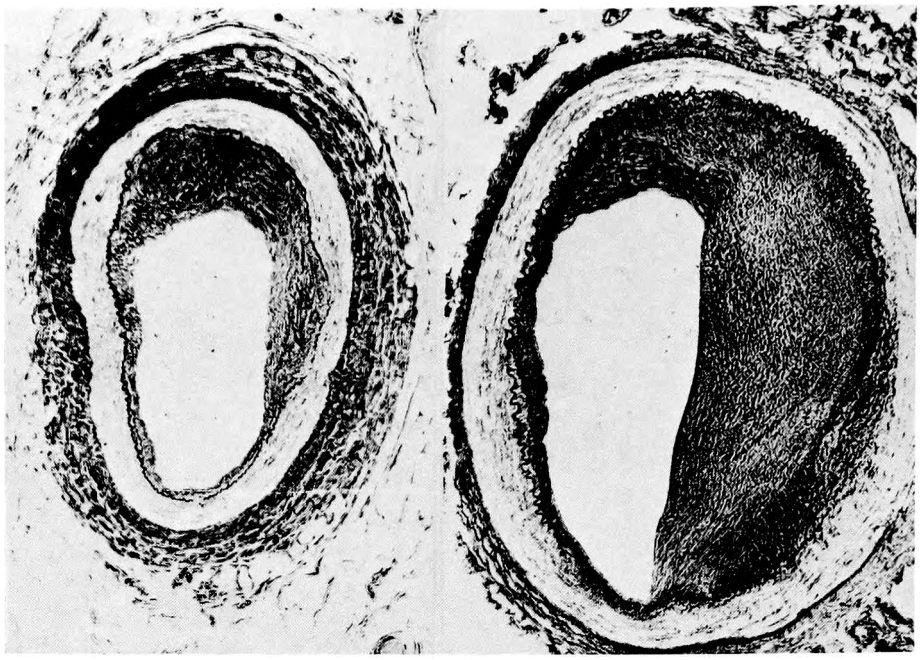


FIGURE 74. (Left) Intimal thickening in the intermediate segment of the right coronary artery of a 12-year-old juvenile with the common type of distribution of the coronary arteries. (Right) Fibromuscular plaque in the intermediate segment of the right coronary artery of a 14-year-old juvenile with an excessive development of the right coronary artery associated with an underdeveloped left circumflex artery. (Resorcin fuchsin-alcian blue. Magnification × 80.)

these patients all four major coronary arteries were narrowed to this extent.[46] In the great majority of coronary artery samples, the lesions were lipid-free, including a combination of Hurler cells, granular cells, collagen, and ground substance. The severity of coronary atherosclerotic involvement of some children with Hurler's syndrome can be compared with that of adult patients with unstable angina pectoris. In two cases in which we investigated the whole coronary arterial tree, we found 14 atherosclerotic plaques, but we were unable to discover intermediate, transitional stages from fatty streaks or other precursors to mature plaques. All these plaques seemed to develop on their own, starting from a fibromuscular nodular proliferation which occurred within branch pads or cushions or within diffuse thickened intimas. *The Hurler syndrome offers adequate material to demonstrate that atherosclerotic plaques do not require more than a few years to progress from early to advanced forms; they do not require precursors for their onset and progression.*

Hurler's syndrome is not the unique condition in which coronary atherosclerotic plaques develop and progress very rapidly in the coronary arteries of children in the absence of the major risk factors for coronary heart disease. As noted before, a demonstrative example is also Kawasaki disease, a mucocutaneous lymph node syndrome in which stenotic plaques, usually lipid-free, made their appearance in the coronary arteries of children. We have also observed the rapid onset and development of plaques related to the presence of some associated diseases, as well as related to particular patterns of hemodynamic stresses (Figure 74).

In children with familial hypercholesterolemia, a very rapid onset and progression of coronary atherosclerotic plaques can be seen in the coronary arteries, but these lesions are lipid-rich, including both lipid-laden cells and extracellular lipid accumulations. Heterozygotes for familial hypercholesterolemia (about 1 in every 500 children) have one normal gene and one abnormal gene for the LDL receptor.[110] With only half of the number of LDL receptors present on the cell surface, LDL particles are removed from plasma at only two

thirds the normal rate. This leads to activation of the alternative receptor-independent pathway which is not associated with inhibition of LDL receptor synthesis. Both overproduction and inefficient catabolism of LDL are followed by a sustained two- to threefold elevation in plasma LDL values since the time of birth. In familial hypercholesterolemia homozygotes, a six- to eightfold elevation in plasma LDL levels can be detected as early as the 20th week of intrauterine gestation. The changes which occur in the coronary arteries of children were compared with those produced in Watanabe's HHL rabbits.[111] The earliest detectable lesion seems to be a deposition of cholesteryl esters in smooth muscle cells and macrophages of the intima and the media. With time, the lesion develops into a full-blown atherosclerotic plaque with a necrotic cholesteryl-ester filled core and a fibrous cap. In spite of the fact that the prevalent pathologic change is lipid overload and occurrence of lipid-laden cells, we could not find in the available literature microphotographs of intermediate, transitional stages between fatty streaks and fibrous plaques. In our cases with familial hypercholesterolemia, the fibronecrotic plaques present in the coronary arteries of children, juveniles, and young adults seem to develop on their own (without precursors), as in Hurler's syndrome. The difference recorded resides mainly in the accumulated material: cholesteryl esters or dermatan sulfate-heparan sulfate.

C. Gelatinous Lesions and Intimal Necrotic Areas

The occurrence of gelatinous lesions and intimal necrotic areas in the coronary arteries of children is strongly related to the mechanism of insudation. It was first involved in atherogenesis by German pathologists[112-118] who consider that insudation may act as a serous inflammation able to produce severe vascular injuries. A disruptive serous inflammation may be followed by the occurrence of "Quellungsnekrose", a replacement of the intimal microarchitecture by a stainless, necrotic material.[118] During the last decades, few pathologists and biochemists have attempted to draw attention to the role of insudation in atherogenesis, assuming that atherosclerotic plaques develop on a plasmogenic base; intimal insudation would produce edema and necrosis, occurring as gelatinous areas, gelatinous thickenings, gelatinous lesions, gelatinous plaques, gray gelatinous elevations — all acting as possible precursors of advanced atherosclerotic lesions.[31,119-126] In essence, certain stenotic or occlusive plaques present in the coronary arteries of mature adults might have their roots in pathological changes produced by insudation in the coronary arteries of children and which occur as gelatinous lesions or intimal necrotic areas.

Gelatinous lesions of the coronary arteries of children cannot be detected on gross inspection, but only on light microscopic examination (Figure 75). In line with this assumption is the view that "although the gelatinous lesions are probably the most significant early lesions, their measurement on an epidemiological scale present serious difficulties".[127]

In the coronary arteries of children, even the use of a hand lens may not facilitate the identification of gelatinous lesions and intimal necrotic areas. Only the larger ones may appear as a blister when the diameter reaches 0.5 to 0.8 mm. Alternatively, they can easily be detected microscopically on longitudinally cut sections or successive cross-sections when they appear as areas of uniform swelling within the thickened intima, or as focal intimal edema prevailing in the subendothelial zone. The separation of intimal fibers by edema is associated with a mucoid appearance, with absolute or relative diminution of the ground substance and fiber histochemical reactivity (Figure 76). In a typical gelatinous lesion, loosely packed collagen fibers are present between rather sparse smooth muscle cells. Some lesions contain large pools of plasma insudate, others densely packed cells and fibers. Finally, in certain lesions the depletion of the ground substance and disintegration of collagen and elastic fibers prevail. When these changes led to the aspects of dissecting necrosis associated with degenerative cell changes, a transition from a gelatinous lesion to an intimal necrotic area was recorded. This necrotic material provided a large pool of substrate in which lipid may

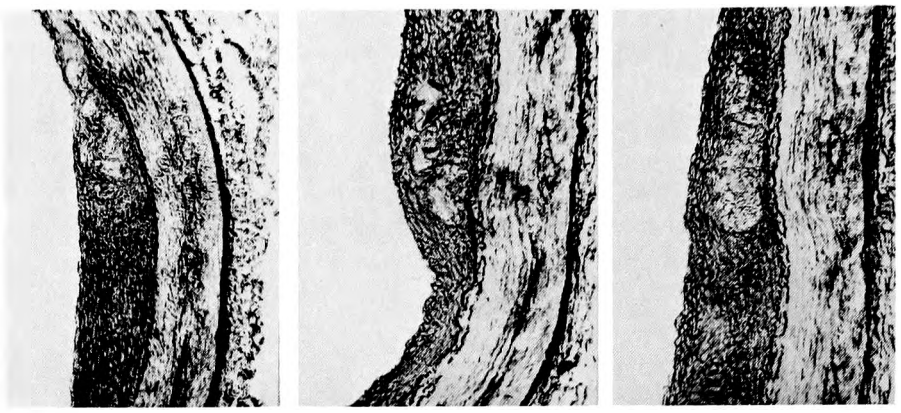


FIGURE 75. Gelatinous lesions detected only on light microscopic examination within the thickened intima of the proximal segment of the left anterior descending artery of juveniles 12 years old. On gross inspection, these lesions were recorded as normal intima. (Resorcin fuchsin-alcian blue. Magnification × 80.)

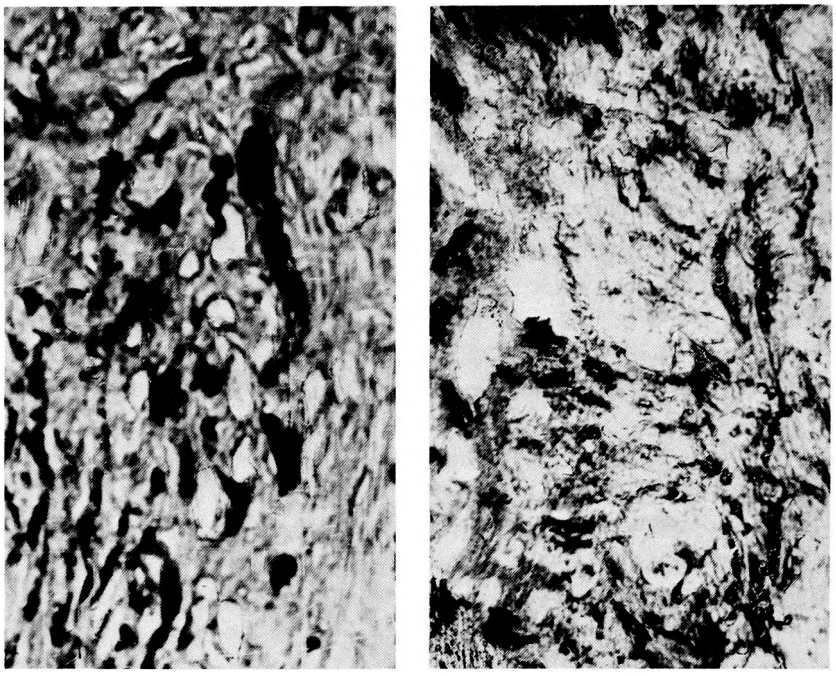


FIGURE 76. Disintegration of fibers (left) and depletion of the ground substance (right) in gelatinous lesions of children. (Resorcin fuchsin-alcian blue. Magnification × 440.)

be deposited and could act as a developing necrotic center of an advanced fibronecrotic plaque. When the gelatinous lesion remained as such, fat was usually present only along the internal elastic membrane or thick collagen fibers. With special stains, fibrin was also detected in gelatinous lesions.[31] On electron microscopic examination, fat droplets or small osmiophil bodies were rarely seen.

Certain fatty streaks may develop in the close vicinity of preexistent gelatinous lesions (or vice versa) and a sharp delineation could be made between these two types of early atherosclerotic lesions. We also revealed gelatinous lesions developed in the basal intimal region, whereas in the subendothelial area a fatty streak was present (Figure 77). On gross

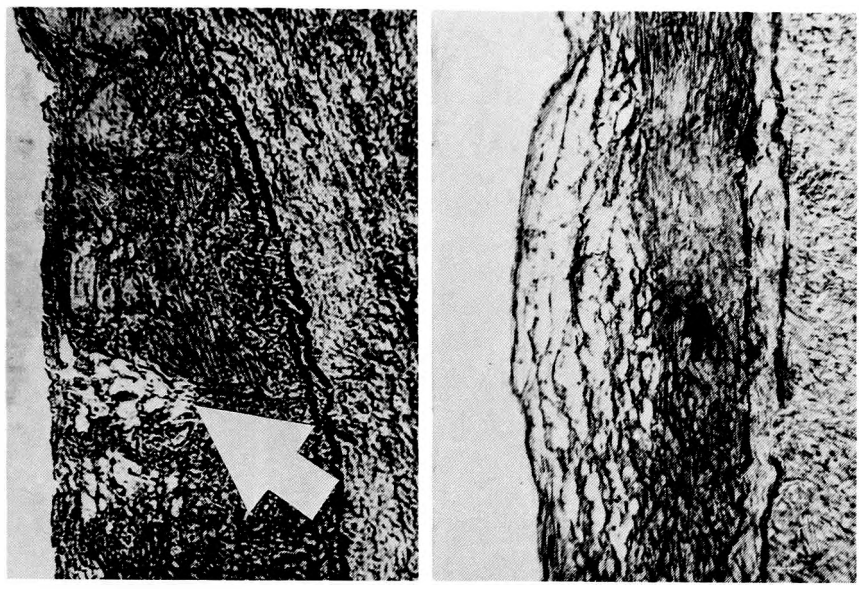


FIGURE 77. Nonbranched region of the proximal segment of the left anterior descending artery of a 13-year-old juvenile. Both fatty streaks (white arrow) and gelatinous lesions are present, but on gross inspection only the fatty streak was recorded. On the right side of the microphotograph, a nonbranched region of the intermediate segment of the right coronary artery of a 19-year-old adolescent is presented. Gross inspection revealed only the presence of a fatty streak, whereas on light microscopic examination, the fatty streak is superimposed on a gelatinous lesion (black arrow) which occurs covered by clusters of lipid filled cells. (Resorcin fuchsin-alcian blue. Magnification × 440.)

inspection only the fatty streaks were recorded and the areas with gelatinous lesions were considered normal intima.

In our material, the first gelatinous lesions were detected in the coronary arteries of children more than 5 years old in the proximal segment of the left anterior descending artery at 1 to 1.5 cm from the bifurcation area of the left main coronary artery. They showed the light microscopic feature of mucoid and swelling necrosis and the incidence was 2% in the age subgroup 6 to 10 and 6% in the age subgroup 11 to 15 years old (Figure 78). During adolescence, gelatinous lesions were also present in the proximal segment of the circumflex artery and in the proximal and intermediate segments of the right coronary artery. In certain adolescents, gelatinous lesions developed first in the segment of the right coronary artery located between the branch site of the right marginal and posterior descending vessels.

The nature of plasma histolytic agents which produce gelatinous lesions and intimal necrotic areas remains a subject of speculation. Some gelatinous lesions may contain less LDL than blood plasma,[127] and this might suggest that the LDL present in such lesions is not intrinsically atherogenic.[128] Moreover, when in gelatinous lesions smooth muscle cell proliferate, this proliferation was not necessarily associated with an increase in LDL concentration. As the early lesion progresses toward more advanced forms, a higher concentration of macromolecules appears in the interstitial fluid. An 11-fold difference could be detected in this respect between an early and advanced lesion: *LDL, fibrinogen, and other macromolecules appear concentrated to a similar extent and this might represent a main mechanism involved in the formation of fibronecrotic or fibrohyaline plaques starting from gelatinous lesions.*[128] Redistribution of tissue water, which appears to shift out of the interstitial fluid compartment, may lead to extraordinarily high concentrations of large macromolecules in localized areas of the interstitial fluid, thereby increasing the probability of their extracellular deposition.

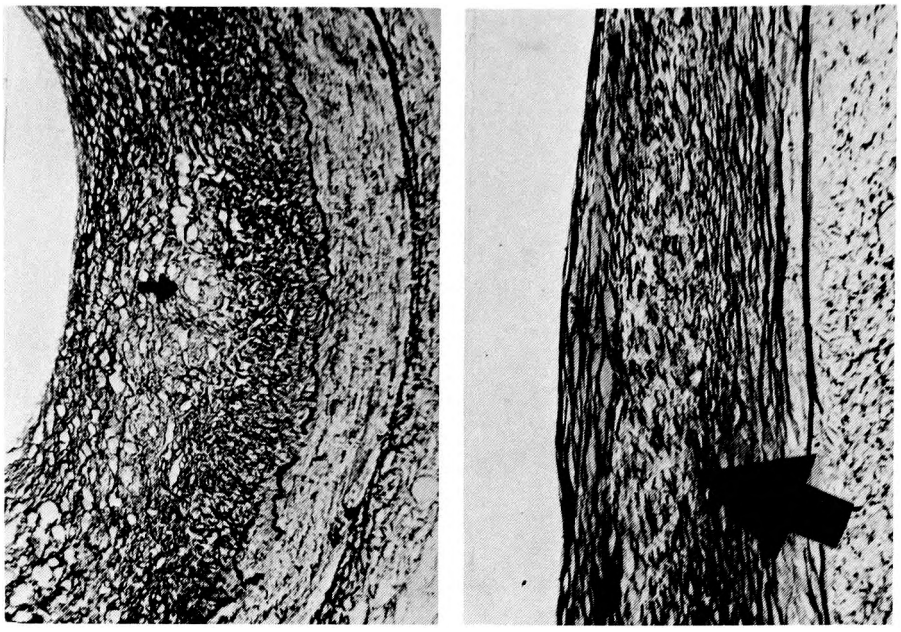


FIGURE 78. Proximal segment of the left anterior descending artery in two juveniles 12 and 14 years old. (Left) Typical gelatinous lesion (small arrow) developed within the very thick intimal layer as a lesion rich in edematous and mucinous material. (Right) Typical intimal necrotic area (large arrow) developed within the very thick intimal layer as a lesion in which the intimal matrix disintegrated. (Resorcin fuchsin-alcian blue. Magnification × 220.)

D. Fatty Streaks

Many data of this subject have already been presented in Sections III.D and III.E.

The first fatty streaks were detected in our material in children and juveniles aged 11 to 15 years in only 16% of individuals, whereas in the aortic valve ring region fatty streaking showed an universal character. It is without doubt that in the aorta fatty streaks are the first atherosclerotic lesions, whereas in the coronary arteries they lag behind the first fibromuscular plaques and gelatinous lesions by 5 to 8 years; likewise in our studies, fibromuscular plaques, gelatinous lesions, and fatty streaks developed in the same period of life (young adulthood) in the intracranial arteries.

In the unselected Bucharest population sample, fatty streaks did not occur as the most important early atherosclerotic lesion, this role being attributed to fibromuscular plaques. In other studies and population groups fatty streaks are presented as the unique early lesions which are the precursors of fibrous plaques. This conclusion seems inevitable if only gross inspection is used to detect atherosclerotic lesions, since fibromuscular plaques, gelatinous lesions, intimal necrotic areas, incorporated microthrombi, and intramural thrombi can be seen only by light microscopy.

An interesting observation is that in certain population groups and races the extent of coronary artery fatty streaks at 15 to 39 years of age did predict the extent of coronary raised lesions at 45 to 54 years of age.[11] The results of the International Atherosclerosis Project maintain that fatty streaks in the coronary arteries parallel raised lesions in older persons both on a group and on an anatomical basis and that they may have some predictive value for the severity of advanced atherosclerosis later in life.

Perusal of the literature demonstrates that the first fatty streaks were rarely detected in the coronary arteries of European and North American children less than 10 years old; on the other hand, in a study carried out on children in Tokyo, the incidence of fatty streaks was very high, namely 25% of cases up to 10 years old.[30] These differences refer to only fatty streaks visible on gross inspection; if more refined methods are used, lipid-laden cells

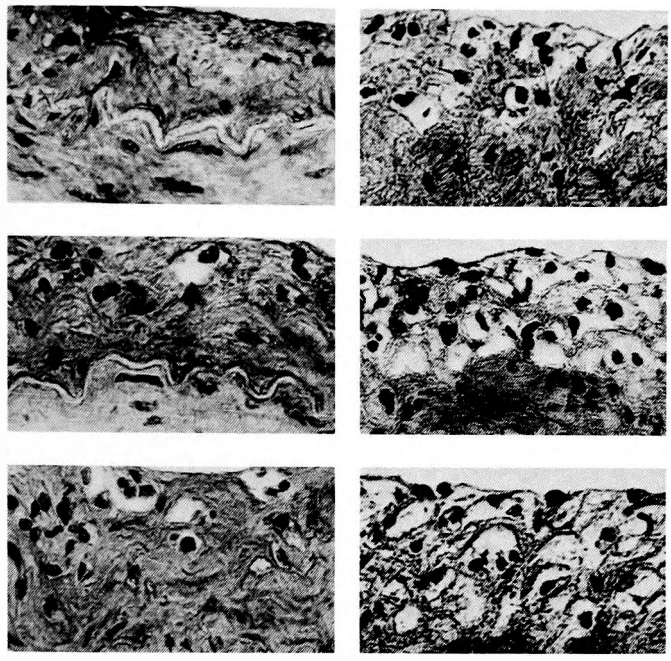


FIGURE 79. Similar topographic site, located 15 mm from the origin of the left anterior descending artery in juveniles aged 11 to 15 years. Suggested intermediate, transitional stages leading to the occurrence of a fatty streak. (Resorcin fuchsin-alcian blue. Magnification × 220.)

can be found in the coronary arteries starting from newborn babies. In the New Orleans population, small collections of intimal macrophage foam cells could be revealed as early as the first month of life. The earliest accumulations consisted of small groups of macrophage-foam cells floating in the glycosaminoglycan-rich matrix of the thickened coronary intima.[51] Unaccompanied by lipid-laden smooth muscle cells or by extracellular debris, the foam cell aggregates are considered to represent the first chapter in the long story of human atherosclerotic involvement.

The progressive accumulation of these clusters of lipid-laden cells leads to the onset of fatty streaks, dots, and spots visible on light microscopic examination and on gross inspection after Sudan staining. Our studies show that the first fatty streaks visible on gross inspection develop in the unbranched region of the proximal segment of the left anterior descending and left circumflex arteries and of the proximal and intermediate segments of the right coronary artery (Figure 79). The topography of many fatty streaks was similar to that of gelatinous lesions which also develop in nonbranched regions and spare branch sites.

The use of the method of successive observations of similar topographic sites of the coronary arterial bed placed in sequence according to age, sex, and anatomical branching pattern allowed us to reveal that in areas where fatty streak usually develop, their onset was preceded by particular changes. The areas where the first collection of lipid-filled cells were detected in children and juveniles aged 11 to 15 years and appeared in children 6 to 10 years old submitted to the action of an insudate, which may act in certain sites as a histolytic agent. This led to progressive replacement of a part of the ground substance and fibers by a mucinous material, sometimes similar to that found in early gelatinous lesions. It exhibited weak toluidine blue basophilia, stained bluish-green with alcian blue and was faintly positive with the PAS technique. This material was partly susceptible to sialidase, collagenase, and chondroitinase ABC digestions. Some of these areas including mucinous material remained as such, giving rise to gelatinous lesions, whereas others showed abundant accumulation of smooth muscle cells and monocyte-macrophages which progressively acquired the light

FIGURE 80. Aorta (left) and the left anterior descending coronary artery (right) of a 12-year-old juvenile. Serially cut sections. Presence of the central mass of foam cells in an abundant mucinous material. (Resorcin fuchsin-alcian blue. Magnification × 120.)

microscopic feature of foam cells. They accumulated in clusters within the subendothelial region and could be visualized as fatty streaks, dots, spots, patches, etc., able to stain distinctly with Sudan dyes. Serial sections in such lesions revealed at their periphery focal insudation, then small areas in which both ground substance and fibers were disintegrated, and finally foam cell accumulations. In certain lesions, as the actual site of the fatty streaks was approached in serially cut sections, more numerous and large foam cells appeared in a mass of mucoid necrosis similar to that seen in gelatinous lesions (Figure 80). To sum up, in many of our successive observations of similar topographic sites placed in sequence according to age, intimal areas with histolysis seemed to develop first, followed by accumulation of smooth muscle cells and monocyte-macrophages which became foam cells. There are grounds for speculation that lipids accumulate in certain smooth muscle cells agglomerated in areas of intimal insudation because these cells are injured and maintained in an inadequate environment. Intracellular fat accumulation is a common reaction of many cells to injury.

On the other hand, the results of experimental studies show that the main precondition for the onset of fatty streak-like lesions is abnormal accumulation of free cholesterol and cholesterol esters in the subendothelial area. Therefore, some lesions called fatty streaks may reflect the existence of an active lipid clearing system involving monocyte-macrophages and are, in fact, no lesions at all; on the contrary, they may be regarded as antiatherogenic response of the reticulo-endothelial system to intraarterial lipid accumulation. If fatty streaks are subendothelial changes induced by hypoxic conditions, toxic influences, altered metabolism, scavenger function of monocyte-macrophages, and many other mechanisms, the attempts to quantitate all fatty streaks considered as homogeneous atherosclerotic lesions may lead to debatable results. Of particular interest is the observation that a number of children, having died during the postwar famine, had been severely emaciated as a result of starvation; however, they showed no difference in the incidence of extent of fatty streaks as compared with well-nourished children.[129]

"Controversy still clouds the relationship, if any, that may exist between the fatty streak and the raised fibrolipid plaque, which is universally accepted as the true lesion of atherosclerosis."[130] Part of this difficulty is considered to reside in the heterogeneity of lesions called fatty streaks.

According to certain views,[131] it is possible to differentiate at least three types of fatty streaks:

1. Those streaks occurring predominantly in childhood and adolescence and which are found in all population groups, socioeconomic circumstances, and susceptibility of the population to develop advanced atherosclerotic lesions and myocardial clinical manifestations. These fatty streaks of children and adolescents are considered without important influence on the natural history of coronary atherosclerosis. In such lesions, the lipid is predominantly intracellular, there is little or no formation of new connective tissue, and there are no extracellular lipid deposits.
2. A second type of fatty streaks was detected mainly in young adults, especially in those who belong to population groups in which there is a high background level of coronary atherosclerosis and high frequency of myocardial clinical manifestations. This type of lesion contains much of its lipid as extracellular accumulations which are found in areas where intact cells are scanty; in other areas numerous cells, both of smooth muscle and monocyte-macrophage origin, are present and some of these cells appear to be undergoing necrosis. An increase in extracellular connective tissue elements is also present. It has been suggested that this type of fatty streak may be progressing and that it may constitute a precursor of the fibrolipid plaque.
3. A third type of fatty streak may be found which occurs chiefly in middle-aged and elderly individuals. In these lesions there is diffuse infiltration of the intima by lipid, fine extracellular droplets of sudanophilic material being concentrated in close apposition to elastic fibers. Cells are scanty and there are no large pools of extracellular lipid. At present, there is no evidence that these lesions undergo transition and grow into advanced plaques.[131]

In certain studies emphasis is placed on the severity of inflammatory cell infiltration and the prevalence of foci of necrosis within the fatty streaks, such changes indicating progression toward advanced plaques.[132] In other studies, the propensity for individual fatty streaks to progress to an advanced form is related to abnormal cellular proliferations of the monoclonal type.[133]

For more than 100 years, this suggested conversion of fatty streaks into fibrous plaques could not be demonstrated by a convincing sequence of microphotographs. Even in an experimental controlled study designed to show fatty streak conversion to fibrous plaques,[134] the lack of microphotographs consistent with the demonstration of this conversion invites the reader to deduce it from the dynamics of events shown diagramatically.

If this conversion really exists, many intermediate, transitional stages must also exist between a fatty streak and a fibrous plaque, but they were not as yet identified by us and by others in successive age groups from childhood to adulthood.

In the coronary arterial trees of various populations there are thousands of fatty streaks and fibrous plaques; theoretically there would also exist in the major coronary arteries and their branches innumerable intermediate stages of transition between these two types of lesions and it is difficult to explain why we all miss this stepwise transformation photographically. We were able to present a succession of static aspects suggesting the progression of fibromuscular plaques, gelatinous lesions, intimal necrotic areas, incorporated microthrombi, and intramural thrombi toward advanced stenotic or occlusive plaques. On the other hand, important difficulties appeared when we intended to demonstrate that fatty streaks

play a major role as precursors of advanced plaques, but this might be a peculiar feature of the material investigated.

III. ATHEROSCLEROTIC INVOLVEMENT OF THE CORONARY ARTERIES OF ADOLESCENTS AND YOUNG ADULTS

A. The 16 to 20 Years Age Group

Except fatty streaks, all the other early lesions escaped recognition at gross inspection. Consequently, the coronary arteries of adolescents and young adults are less adequate than those of adults for quantitative assessments based on visual inspection. This view is also reinforced by the above-mentioned heterogeneity of fatty streak-like lesions.

Since there are no data in the available literature on light microscopic examinations of the whole coronary arterial tree, our presentation is based on the results obtained in our laboratory.

1. The *atherosclerotic plaques* were present:
   In 1 out of 25 subjects in the 11 to 15 years age group
   In 1 out of 8 subjects in the 16 to 20 years age group
2. The *gelatinous lesions* and *intimal necrotic areas* were present:
   In 1 out of 16 subjects in the 11 to 15 years age group
   In 1 out of 6 subjects in the 16 to 20 years age group
3. The *fatty streaks* were present:
   In 1 out of 6 subjects in the 11 to 15 years age group
   In 1 out of 3 subjects in the 16 to 20 years age group
4. *Intramural thrombi* and *incorporated microthrombi* were present:
   In 1 out of 25 subjects in the 11 to 15 years age group
   In 1 out of 12 subjects in the 16 to 20 years age group

This reflects a considerable extension of coronary atherosclerotic involvement of all types of early lesions from juveniles to adolescents. This extension took place in a population with a total serum cholesterol level of 120 to 160 mg/dℓ and without detectable abnormalities of lipid metabolism with the methods used. Only 3% of these adolescents were overweight and only 3% showed high blood pressure levels.[174] Smoking was present in less than 1/4 of cases. This unselected population sample is very demonstrative as it shows that coronary atherosclerosis may occur and progress in the absence of the major risk factors for coronary heart disease. This absence was associated with smooth muscle cell focal proliferations leading to the onset of fibromuscular plaques, intimal insudation occurring as gelatinous lesions and intimal necrotic areas, intraintimal fibrin and platelet accumulation giving rise to intramural thrombi, and development of lipid-laden cells acquiring the light microscopic feature of fatty streaks. All these early lesions seem to have an independent origin and a particular histogenesis, topography, and rate of progression in agreement with the multifactorial origin of human atherosclerosis.

During childhood, plaques were found only in the bifurcation region of the left main coronary artery and branch sites of the left anterior descending artery; during adolescence they also developed in nonbranched regions and in branch sites of the left circumflex and right coronary arteries. This development appeared in our material strongly related to the anatomical branching pattern which is genetically determined. In subjects in which the right arterial system showed a considerable development which coexisted with a short and narrow left circumflex artery, plaques developed in both the proximal and intermediate segments of the right coronary artery, particularly at the origin of the right marginal and posterior descending branches. In undistended vessels these plaques narrowed up to 50% of the arterial lumen.

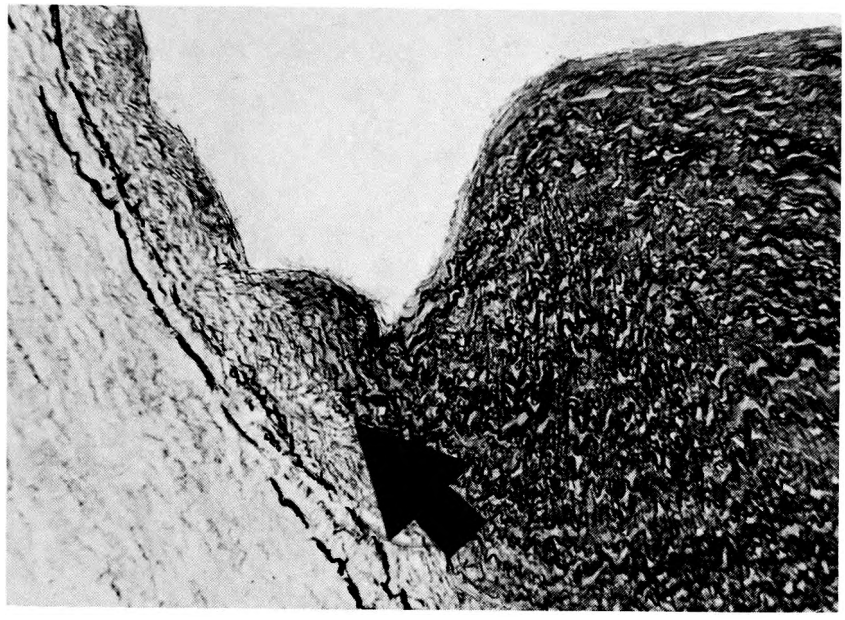


FIGURE 81. A clear border (arrow) separates the thickened coronary intima from the fibromuscular plaque. The intimal connective tissue and the tissue which forms the lesion do not interpenetrate. (Resorcin fuchsin-alcian blue. Magnification × 440.)

These observations demonstrate that *the univascular involvement with atherosclerotic plaques characteristic in children is replaced in adolescents, by a multivessel involvement of the coronary arterial tree.*

A multivessel disease exists starting from subjects aged 16 to 20 years old, but the atherosclerotic plaques are not visible angiographically and on gross inspection. Two major endogenous risk factors seem to be mainly involved in this multivessel involvement by atherosclerotic plaques: male sex and anatomical branching pattern.

During childhood, only one type of atherosclerotic plaque could be revealed by light microscopy: the *fibromuscular* type. In adolescents this type of early lesion was also present and prevalent, exhibiting at its periphery a clean border separating the surface area of the plaque from that of the normal intima (Figure 81). Likewise, in the coronary arteries of adolescents we revealed a particular change in these fibromuscular plaques, characterized by intralesional occurrence of large areas of edema. In these edematous areas we detected fiber disintegration, ground substance depletion, and degenerative cell changes (Figure 82). The term "mucoid plaque" is usually attributed to these fibromuscular plaques including large areas of insudation. The difference between a mucoid plaque and a gelatinous lesion is sometimes difficult to establish; edema which develops in preexisting fibromuscular plaques gives rise to mucoid plaques and edema which develops in preexisting thickened intima gives rise to gelatinous lesions. In both sites, edema may exert only a mechanical, distending effect, or may produce ground substance depletion, fiber disintegration, and cell alterations. If the plasma components which perform the insudate act as necrotizing agents, then the fibromuscular plaque is converted to a necrotic plaque and the gelatinous lesion to an intimal necrotic area. For reasons which are not clear, this necrosis became prevalent in our material in young adults and not in adolescents in which the mucoid aspect remains prevalent. In intracranial arteries, the onset of mucoid plaques was detected in the basilar artery in subjects aged 31 to 35 years and in the anterior cerebral artery in subjects aged 41 to 45 years.[7]

In the great majority of mucoid plaques, in both coronary and intracranial arteries, we found scattered lipid-filled cells, particularly in the subendothelial area of the lesion. We

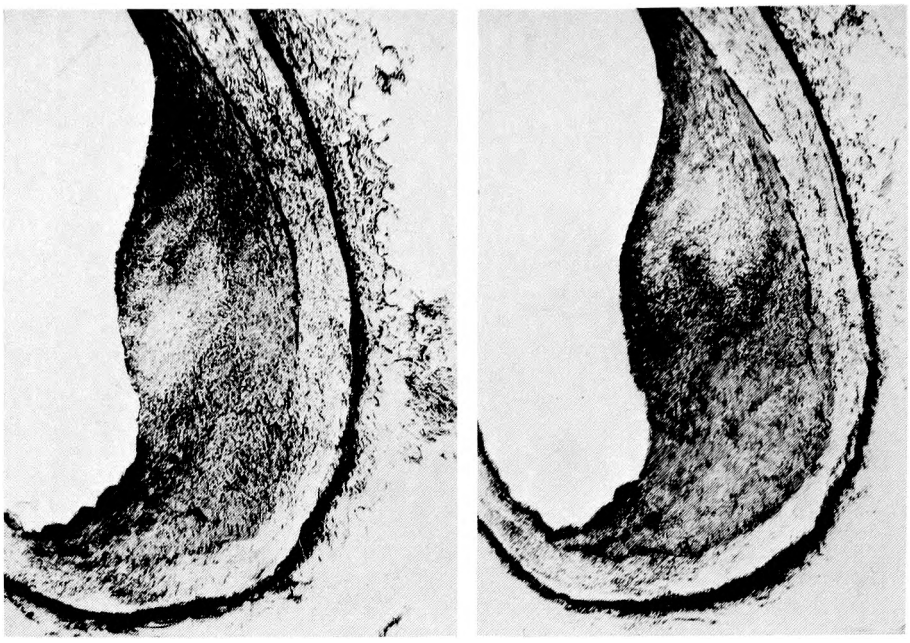


FIGURE 82. Proximal segment of the left anterior descending coronary artery of a 16-year-old adolescent. Two cross-sections of a fibromuscular plaque separated by a distance of approximately 300 μm, showing the presence of a large area of insudation within the lesion which progressively acquires the light microscopic feature of a mucoid plaque. (Resorcin fuchsin-alcian blue. Magnification × 120.)

could not detect important extra- or intracellular lipid accumulations; likewise, we were unable to reveal the presence of fibrin deposits or of agglomerations of platelets.

Also of note is the observation that fibromuscular plaques developing at branch sites are not involved by insudation and edema and are not converted to mucoid plaques; on the other hand, the fibromuscular plaques developing in unbranched segments are submitted to edema and insudation and acquire the light microscopic feature of mucoid plaques. Likewise, note the observation that in adolescents we could relate the onset of both mucoid plaques and gelatinous lesions to tobacco smoking.[73]

The fatty streaks were the atherosclerotic lesions which, in our studies, showed the most dramatic increase from children and juveniles to adolescents, being detected in only 6% of subjects 11 to 15 years old and in 34% of subjects 16 to 20 years old. Like the above-mentioned insudative phenomena, the clusters of lipid-laden cells usually spared coronary branch sites, particularly those with a branch mouth diameter less than 2 mm. Serial sections revealed that lipid was only intracellular; it stained red-brown and black with the OTAN method and violet with the PAN technique, a moderate Schultz reaction also was observed. In all developing fatty streaks, we recorded histochemically a first stage with a prevalence of phospholipids, particularly sphingomyelin, followed by a progressive appearance of cholesterol esters. We could not detect in the post-mortem material and with the methods used the proportion of smooth muscle cells and monocyte-macrophages lipid-laden cells in each fatty streak-like lesion; we were also unable to appreciate what proportion of cells accumulate lipid as a result of the injury produced by insudation.

The frequency of subjects with microthrombi deposition on the thickened intima and incorporation within this thickened intima increased twofold as compared to the previous age group. Our material, removed 12 to 24 hr after death, is not adequate for correct estimation of this prevalence, as well as that of massive platelet aggregations. The presence of coronary microthrombi and intramural thrombi in only 8% of cases must be considered an underestimation.

In a special study on the discrepancies between data furnished by gross inspection and light microscopy on atherosclerotic involvement of human coronary arteries,[135] we showed that *of the 100 areas recorded as normal intima on gross inspection of the coronary arteries of adolescents, only 69 were also normal histologically.* The remainder included 5 areas with intimal fibrosis, 11 areas with gelatinous lesions and intimal necrosis, 5 areas with incorporated microthrombi, and 10 areas with fibromuscular and mucoid plaques.

This would mean that approximately 1/3 of the intimal surface considered to be normal on gross inspection is in fact, not normal, including many types of early atherosclerotic lesions visible only on light microscopy.

B. The 21 to 25 Years Age Group

In this age group, including very young adults, atherosclerotic plaques, gelatinous lesions, intimal necrotic areas, and very thick intimas were detected in one out of every three individuals, fatty streaks in one out of two and microthrombi in one out of seven.

Compared with the percentage of cases with atherosclerotic lesions in the age group 16 to 20 years, the proportion of subjects with atherosclerotic plaques increased from 12 to 28%, the proportion of subjects with gelatinous lesions and intimal necrotic areas from 16 to 32%, the proportion of subjects with fatty streaks from 34 to 52%, and finally, the proportion of subjects with incorporated microthrombi and intramural thrombi from 8 to 14%.[13,73]

Since not all these types of early lesions were present in the same coronary arterial bed (some subjects having only fatty streaks, others only atherosclerotic plaques or gelatinous lesions), it might be assumed that starting from this age group, each individual has in its coronary arterial tree at least one type of early atherosclerotic lesion. *Histologically, it is not possible to maintain that there are subjects free of atherosclerotic involvement starting with the third decade of life.* On the other hand, on gross inspection, only fatty streaks were usually recorded. Of 100 areas considered grossly normal intima, we found on light microscopic examination a correspondence in 53% of cases. The remaining 47% of apparently normal intima included 20% areas with gelatinous lesions and intimal necrosis, 15% areas with nonraised atherosclerotic plaques, 6% areas with the incorporated microthrombi or intramural thrombi, and 6% areas with diffuse intimal fibrosis.

It is worth mentioning that whereas some of the fibromuscular and mucoid plaques were grossly recorded by us as normal intima, the foam cell-rich plaques, characteristic of the coronary arteries of young adults, might be erroneously recorded as fatty streaks, since they are not raised lesions. The light microscopic examination of lesions grossly recorded as fatty streaks in young adults showed that 15% of these lesions were in fact foam cell-rich plaques.[135] From 28 plaques detected in 12 subjects 16 to 20 years old, 10 were of fibromuscular and 18 of mucoid type. From 59 plaques detected in 28 subjects 21 to 25 years old, only 6 were of fibromuscular type, 6 of the mucoid type, 12 of the foam cell-rich type, and 4 of the necrotic type. This means that starting from the third decade of life, we could differentiate histologically in the coronary arterial tree at least four types of plaques. *Whereas the prevalent plaque during childhood is the fibromuscular, during adolescence the mucoid plaque becomes prevalent and in early adulthood the foam cell-rich plaque.* We proposed the term "foam cell-rich plaque" to designate a mucoid plaque with many clusters of lipid-laden cells.[73] In our material, the occurrence of lipid-laden cells was not associated with an augmentation in serum cholesterol level or of LDL-cholesterol and could not be related to particular diets rich in cholesterol and saturated fats or with other detectable lifestyle changes. In fact, the respective occurrence of clusters of lipid-laden cells in preexisting fibromuscular and mucoid plaques was limited only to the unbranched regions of the proximal segments of the major coronary arteries and could be revealed only in young adults, its significance as related to age, arterial bed, and vessel segment being obscure. This accumulation of lipid-

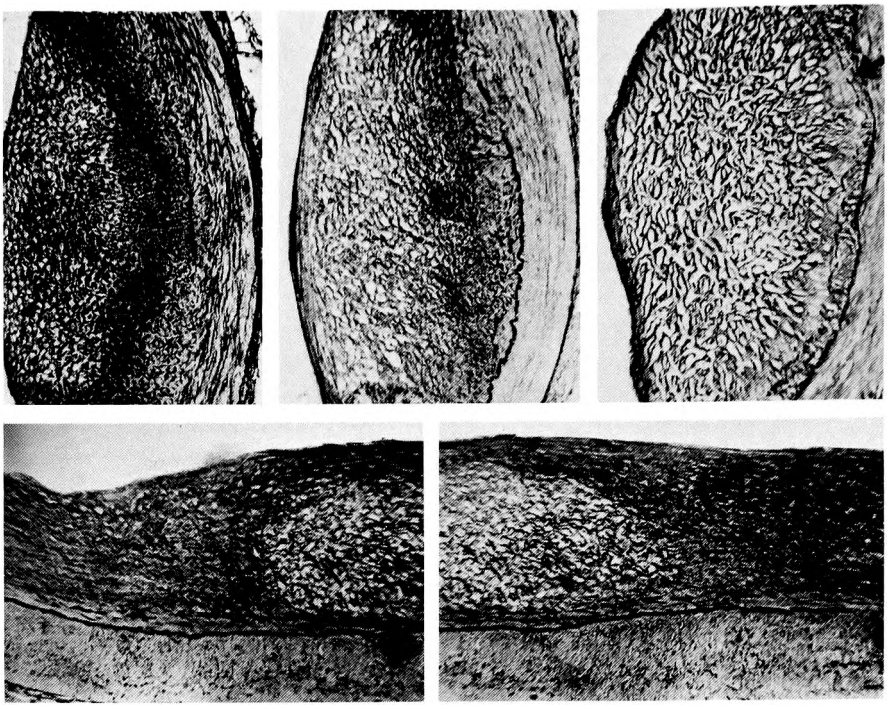


FIGURE 83. Foam cell-rich plaques. (Top) Cross sections, (bottom) longitudinal section. Nonbranched region of the proximal segment of the major coronary arteries of young adults 21 to 30 years old. (Resorcin fuchsin-alcian blue. Magnification × 120.)

filled cells in preexisting mucoid plaques was not associated with a similar occurrence of clusters of lipid-laden cells in preexisting gelatinous lesions and did not appear associated with a dramatic increase in the number of subjects with coronary fatty streaks (this proportion augmented from 52% in subjects 21 to 25 years old to 66% in subjects 26 to 30 years old). Some investigators were also able to demonstrate the existence of a sudanophilic plaque rich in lipid-filled cells, which seems to precede the onset of advanced lesions.[136]

In young adults we also found a particular type of atherosclerotic plaque, the necrotic.[14,137] This term was attributed to fibromuscular, mucoid, or foam cell-rich plaques in which a large basal necrotic center developed, not covered by a fibrous cap. This type of lesions will become prevalent in mature adults, showing a fibrous cap and acquiring the macroscopic feature of the fibrous plaque and the light microscopic feature of the fibronecrotic plaque. After the onset of the first necrotic plaques in subjects aged 21 to 25 years, both fibrin and lipid accumulate in the basal region of the lesion.

Histologically, the cells of foam cell-rich plaques strongly resembles smooth muscle cells overloaded with lipid droplets. In some of these lesions the agglomeration of lipid-filled cells in frozen sections or of foam cells in paraffin-embedded material exhibited an unexpected density (Figures 83 and 84). In four such subjects with a very high density of lipid-laden cells in foam cell-rich plaques (as illustrated in Figure 84), the level of total serum cholesterol was 138, 147, 172, and 174 mg/dℓ with an unaltered lipoprotein pattern. In the basilar, vertebral, anterior cerebral, renal, and mesenteric arteries of young adults, we were unable to detect the onset of foam cell-rich plaques. As concerns the major coronary arteries, this onset was restricted to unbranched regions and a fibromuscular plaque developed at a branch site was never seen to give rise to a foam cell-rich plaque. Successive observations of similar topographic sites placed in sequence revealed that the onset of clusters of lipid-filled cells was preceded by focal insudation in a preexisting plaque. Some types of insudation were associated with a massive agglomeration of smooth muscle cells in the preexisting

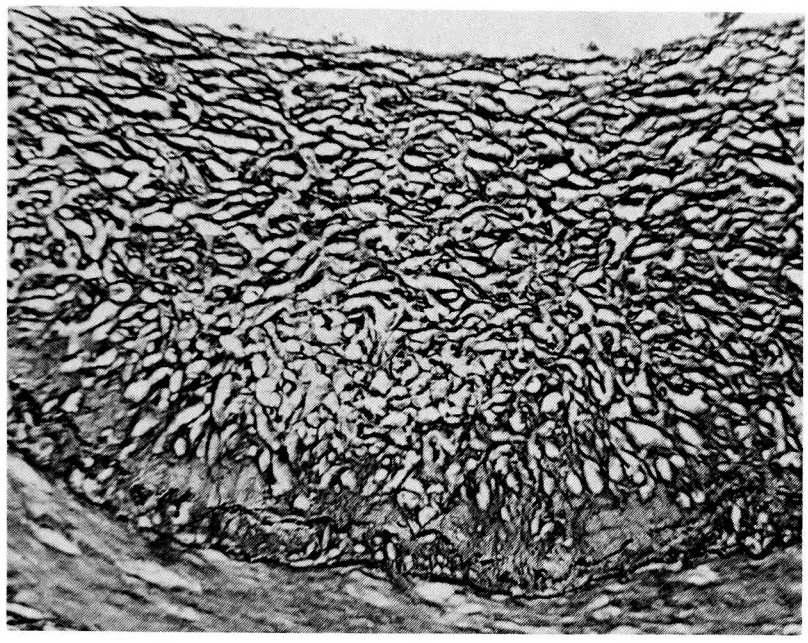


FIGURE 84. Foam cell-rich plaque present in the intermediate segment of the right coronary artery of a 25-year-old young adult. Very high density of foam cells throughout the lesion and the presence of small areas of mucoid necrosis in the basal area. (Resorcin fuchsin-alcian blue. Magnification × 440.)

plaque in which phospholipids and then cholesteryl esters progressively accumulate. The foam cell-rich plaques with abundant intracellular accumulation of cholesteryl esters were progressively capped with newly formed collagen fibers, occurring as an immature fibrous cap. In 12% of young adults 21 to 25 years old, the foam cell-rich plaques appeared involved by small basal areas of swelling necrosis (see Figure 84).

Essentially, the major differences in atherosclerotic involvement between children, on the one hand, adolescents and young adults 21 to 25 years old, on the other hand, consisted of:

- A 3- to 14-fold increase in the number of subjects with atherosclerotic plaques
- Plaque development in unbranched segments of the major coronary arteries leading to a multivascular atherosclerotic involvement
- The association of proliferative with insudative and necrotic changes, leading to the onset of mucoid plaques
- The occurrence of plaques able to encroach upon more than 50% of the vessel lumen (undistended artery)

An outstanding observation related to our study on the coronary arteries of adolescents and young adults is that necrosis, detected in some mucoid plaques and gelatinous lesions, did not result from the break-up of lipid-filled cells since such cells are usually absent in fibromuscular and mucoid plaques and in gelatinous lesions. This would indicate that this type of necrosis has nothing to do with that obtained in experimental studies in animals submitted to cholesterol-rich diets. In our studies, necrosis seems to develop as a result of the action of multiple enzymatic-like activities, involving step by step the components of the ground substance, fibers, and cells. These necrotizing agents are present in both blood plasma and necrotic material of atherosclerotic lesions, but their real nature remains the subject of speculation. The possible additional role of infectious diseases and immunologic phenomena cannot be overlooked. Whereas in certain population groups and individuals the

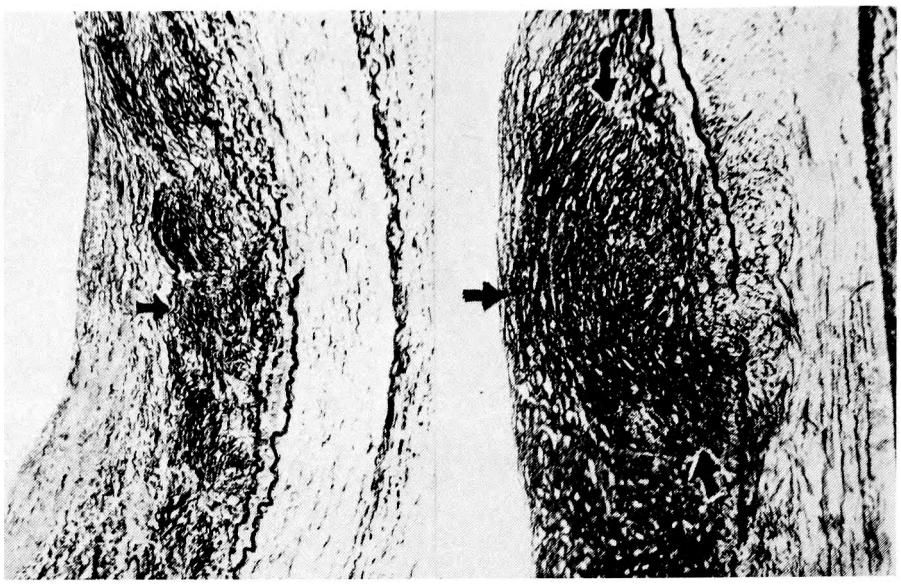


FIGURE 85. Proximal segment of the left anterior descending coronary artery of a young adult. (Left) Small areas of focal proliferation (arrow) in a very thick intima. (Right) Delineation of a fibromuscular plaque (indicated by three arrows) within the thickened intima. (Resorcin fuchsin-alcian blue. Magnification × 220.)

thesauriosmotic aspect of atherosclerosis might prevail, leading to important lipid deposits within the thickened intima, in others the prevalent aspect of atherosclerotic involvement might be that of a necrotizing arteriopathy.

By comparing the frequency of cases with coronary atherosclerotic plaques in children and adolescents, one can deduce that among the 12 adolescents with coronary plaques out of 100 subjects of this age group, only 2 carried these lesions from the age of 6 to 10 years and only 4 from the age of 11 to 15 years. Of 28 plaques detected by light microscopy in 12 adolescents 16 to 20 years old, only 7 (25%) can be considered as initiated in childhood and 21 (75%) might be regarded as newly formed plaques. It also appears clear from our results that plaques developed in the unbranched segments of the proximal segment of the major coronary arteries do not seem related to the atherosclerotic involvement of childhood; they occurred within a short period of 1 to 3 years during adolescence in the thickened intima of the left anterior descending, left circumflex, and right coronary arteries (Figure 85). These fibromuscular plaques developed in nonbranched regions appear more prone to edema, insudation, and necrosis than plaques developed in branch sites. Consequently, the newly formed plaques of adolescents rapidly acquired the light microscopic feature of mucoid plaques, whereas the plaques present in branch sites preserved their initial character of fibromuscular lesion.

By comparing the frequency of cases with coronary atherosclerotic plaques in adolescents and in young adults 21 to 25 years old, we found that among the 28% of young adults with coronary atherosclerotic plaques only 2% carried these plaques from the age of 6 to 10 years, only 4% from the age of 11 to 15 years, and only 12% from the age of 16 to 20 years. The number of newly formed atherosclerotic plaques was deduced by subtracting the percent of subjects with coronary atherosclerotic plaques in a certain age group from the percent of subjects with coronary atherosclerotic plaques in younger age groups. This method led to the conclusion that in 16 out of 28 plaques the development of these lesions started in the third decade of life. The age of certain plaques could also be deduced based on the topography and light microscopic features of the lesion. All plaques present in the proximal segment of the posterior descending and first diagonal arteries can be considered to have occurred in

young adulthood, since their presence was never recorded during adolescence. This would indicate that only 1 to 3 years are necessary for a plaque to develop as a well-delineated lesion and this view is in agreement with many observations carried out on aorto-coronary saphenous vein bypass grafts.

Among other studies carried out on very young adults, the most popular was that performed on U.S. soldiers killed in action in the Korean War.[138,139] It is based on 300 autopsies, in 200 cases the average age being 22 years. In 77.3% of the hearts, some gross evidence of coronary atherosclerosis was found, manifested as fibrous thickenings, streaking, and plaques. True aspects of atheromatous plaques were revealed in more than 42% of cases and other types of changes in 35%. Some plaques produced complete occlusion of one or more vessels.

A second important study was carried out after 18 years on U.S. soldiers killed in action in Vietnam, the average age being also 22 years. This second study revealed "some evidence" of atherosclerosis in 45% of cases, severe atherosclerotic involvement in 5%, whereas a postmortem angiography did not detect narrowings of possible clinical significance.[140] Some discrepancies with the first mentioned study might be related to differences in methodology and also to secular trends in atherosclerotic involvement.[141]

In the International Atherosclerosis Project study, the mean percentage of intimal surface involved with raised lesions varied in the 15 to 24 years age group from 0.3% in Bogota to 2.5 in Caracas and New Orleans Blacks, being 2.1% in New Orleans Whites and 1.9% in Oslo. The analysis of variance does not indicate that the mean value for each location-race group is significantly different from the mean for every other location-race group.[142] At this age period, no clear correlation could be established between the age groups with the highest percentage of intima surface involvement with raised lesions as adolescents and young adults and the subsequent severity of myocardial clinical manifestations during adulthood. In the 15 to 24 years age group, the percentage of cases with fibrous plaques in the left anterior descending artery was 27 for New Orleans Whites and Blacks, 19 for Oslo, 17 for Caracas, 9 for Costa Rica, and 0 for 48 cases from Mexico. Complicated and calcified lesions were absent in the respective age groups.

In a study on coronary atherosclerosis among Hong-Kong Chinese,[143] atherosclerosis was found in three of four young adults, occurring as early lesions consisting of aggregates of foam cells and mild fibrotic reactions. The annual mortality of this population from coronary heart disease was more than three times lower than that of western societies.

Before closing this section, it seems necessary to emphasize that it is very difficult to get a clear idea about the changing pattern of atherosclerotic plaques from a 5-year age group to the next, as it appeared in our material. The fibromuscular plaques accounted for 100% of the atherosclerotic plaques in the 11 to 15 years age group, then decreased to only 33% in the 16 to 20 years age group, and to 21% in young adults 21 to 25 years old. Over a period of only 1 decade, the fibromuscular plaques decreased from 100% to only 21% of the total atherosclerotic plaques. The mucoid plaques accounted for 61% of the total atherosclerotic plaques in adolescents 16 to 20 years old and decreased to only 33% in young adults 21 to 25 years old. The foam cell-rich plaques accounted for only 6% of plaques in adolescents but increased to 42% in young adults 21 to 25 years old. They will disappear in the older age groups, whereas the necrotic plaques which represented only 4% of the plaques in young adults 21 to 25 years old will increase dramatically in mature adults.

C. The 26 to 30 Years Age Group

We were deeply impressed not only by the above-mentioned changing pattern of atherosclerotic plaques from a 5-year age group to the next, but also by the complexity of changes detected in the 26 to 30 years age group in the whole coronary arterial tree. These changes involve proliferation, insudation, intra- and extracellular lipid deposition, mucoid swelling and dissecting necrosis, and focal and diffuse fibrosis associated with accumulation of fibrohyaline material.

The 26 to 30 years age group seems to be the most adequate for demonstrating the existence of intermediate, transitional stages between early and advanced atherosclerotic lesions: conversion of fibromuscular to fibrohyaline or fibronecrotic plaques, conversion of incorporated microthrombi and intramural thrombi to fibrohyaline plaques, and conversion of gelatinous lesions and intimal necrotic areas to fibrohyaline or fibronecrotic plaques. This age group is also adequate for revealing that the passage from a fibromuscular plaque toward an advanced lesion might acquire the succession fibromuscular-mucoid-foam cell-rich-necrotic and finally fibronecrotic plaque, or might appear as a direct transformation of the fibromuscular plaque in a fibrohyaline or fibronecrotic plaque.[17]

In the 26 to 30 years age group, the proportion of subjects with atherosclerotic plaques augmented to 42% compared to only 28% in the preceding age group. Consequently, the ratio of subjects without vs. subjects with atherosclerotic plaques (detected on both gross inspection and light microscopic examination) decreased from 3.6:1 in the 21 to 25 years age group to 2.4:1 in the 26 to 30 years age group, and will continue to decrease to 1.7:1 in the 31 to 35 years age group.

In 42 out of 100 subjects 26 to 30 years old with atherosclerotic plaques we recorded 128 such lesions, the mean serum cholesterol level being 170 mg/dℓ. Only 8% of the subjects belonging to this group had serum cholesterol levels between 220 and 240 mg/dℓ and only 10% were hypertensive, the most important risk factor being tobacco smoking (approximately 1/3 of cases).

In 28 out of 100 subjects 21 to 25 years old with atherosclerotic plaques in the coronary arteries, we recorded 59 such lesions, approximately two plaques per case. In 42 out of 100 subjects 26 to 30 years old with atherosclerotic plaques in the coronary arteries we recorded 128 such lesions, approximately three plaques per case. By subtracting the number of plaques detected in the coronary arterial bed of young adults 21 to 25 years old from the number of plaques detected in the 26 to 30 years age group, it might be presumed that 69 out of 128 plaques were newly formed. This would indicate that more than half are newly formed among the plaques of this age group and that they develop in a short period of 1 to 3 years.

The intense development and rapid progression of atherosclerotic plaques in a population with serum cholesterol levels from 120 to 160 mg/dℓ during childhood and adolescence and from 160 to 200 mg/dℓ during early adulthood suggests that hypercholesterolemia is not a prerequisite for atherosclerotic involvement as it is in experimental models of atherosclerosis. Likewise, arterial hypertension cannot be involved as a major atherogenetic agent, since it was present in less than 5% of young adults. Post-mortem examination of the coronary arteries of men aged more than 25 years revealed a positive association between tobacco smoking and severity of atherosclerotic involvement.[144]

In the 26 to 30 years age group, we found not only an important increase in the number of cases with atherosclerotic plaques, but also a considerable augmentation in the number of cases with involvement of both major coronary arteries and main branch vessels. In addition to left anterior descending, left circumflex, right coronary artery, and left main coronary artery, we revealed atherosclerotic plaques in the proximal segment of the posterior descending, first diagonal, and first septal branches (Figures 86 and 87).

Also of note are the changes in the histological character of plaques, as compared to the 21 to 25 years age group:

- The fibromuscular plaques decreased from 21 to 16% of the total proportion of coronary atherosclerotic plaques
- The mucoid plaques decreased from 35 to 22%
- The foam cell-rich plaques decreased from 42 to 22%
- The necrotic plaques increased dramatically from 4 to 40%

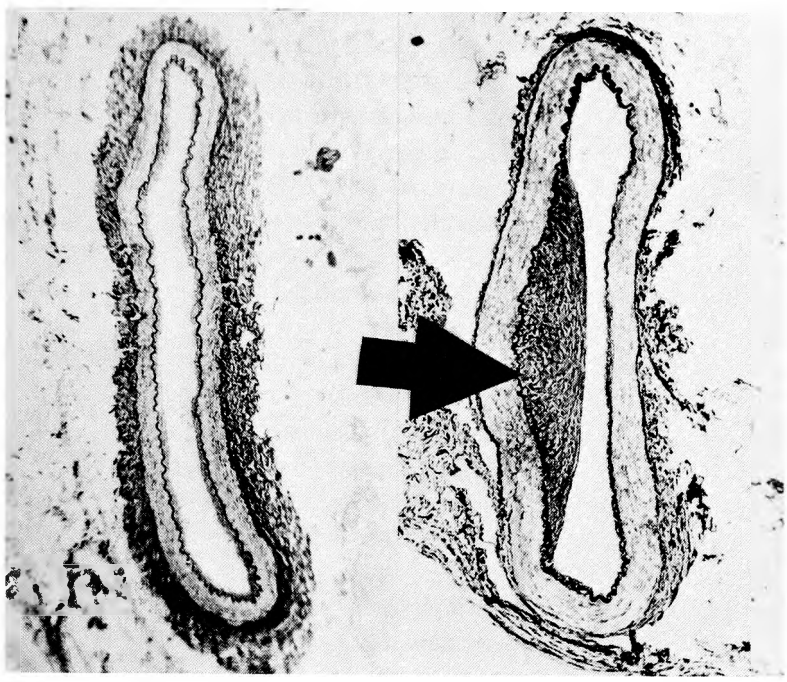


FIGURE 86. Proximal segment of the first diagonal artery arising as a branch of the left anterior descending artery (left) or from the trifurcation of the left main coronary artery (right). In subjects with this last minor deviation from the common type of distribution of the coronary arteries (detected in our material in 6.5% of cases), a thicker arterial wall was present and also fibromuscular plaques (arrow) which may develop in the absence of a diffuse thickened intima. (Resorcin fuchsin-alcian blue. Magnification × 80.)

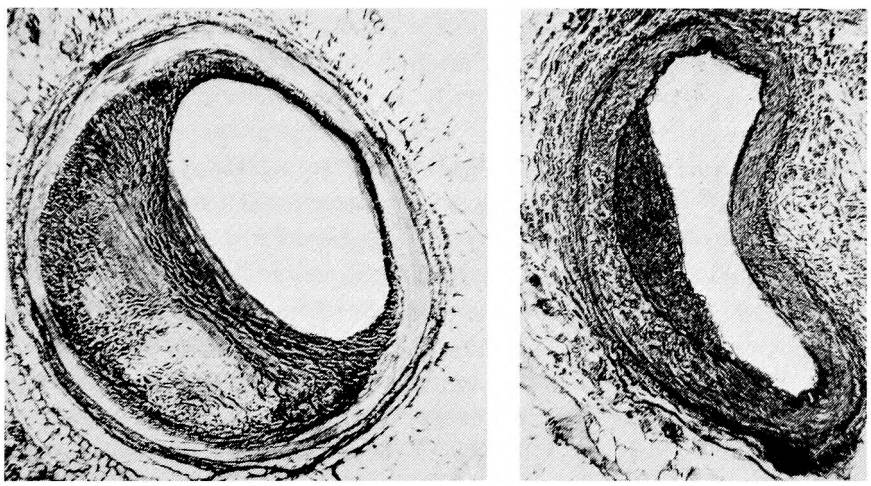


FIGURE 87. The proximal segment of the posterior descending artery arising as a branch of the right coronary artery. In approximately 4% of our unselected cases, this branch showed a considerable development starting from childhood, frequently associated with a short and narrow posterior descending artery (see Figure 43). In 3% of young adults with this deviation we revealed stenotic atherosclerotic plaques (right), whereas in cases with the common type of distribution of the coronary arteries, only focal areas of intimal thickening were detected (left). (Resorcin fuchsin-alcian blue. Magnification × 80.)

During a period of only 5 years, a tenfold increase appeared in the proportion of plaques with large necrotic areas and these lesions became prevalent.

A relation was observed by other investigators between the degree of intimal thickness and plaque necrosis: at the level of an intimal thickness of 0.83 mm, all lesions showed necrosis.[145] This relation did not appear in our studies; we strongly believe that in a certain period of their cycle of evolution toward advanced lesions some plaques and also some regions of the thickened intima are submitted to the action of necrotizing agents present in blood plasma and in intimal insudate. When these necrotizing agents exert an aggressive action, they might transform coronary atherosclerosis from a prevalent proliferative arteriopathy to a prevalent necrotizing arteriopathy.

Concomitant with this marked increase in the number of necrotic plaques, important changes appeared in gelatinous lesions, fatty streaks, and intramural thrombi. Some fatty streaks enlarged and exhibited edema, small foci of necrosis, and lipid-filled cell disintegration, acquiring the feature of an advanced lesion. Some gelatinous lesions showed many areas of swelling necrosis in which the intimal microarchitecture was completely lost and replaced by a finely homogeneous floccular and granular material. The swelling necrosis may occur either as an independent change, or as a precursory step of the dissecting necrosis, in some advanced gelatinous lesions both types of necrosis appearing closely intermingled. Usually the dissecting type of necrosis develops in the basal zone of a gelatinous lesion, leading to a total degradation of the intimal components. The resulting material accumulates in small detritus cavities that coalesce in larger ones, giving rise to lesions similar to necrotic plaques. The absence of any evidence of repair was a striking feature of all forms of transition from gelatinous lesions and intimal necrotic areas to necrotic plaques. Note also that the onset of dissecting necrosis is associated with the appearance of massive lipid deposits, cholesterol clefts, and formation of intramural thrombi. Many samples of the coronary arteries placed in sequence according to age, sex, and anatomical branching pattern suggest that some gelatinous lesions appear first, progress to necrotic plaques, and cause local deposition of lipid and fibrin. Other gelatinous lesions also appear first, but progress directly to fibrohyaline plaques without significant lipid accumulation within the lesion.

In an investigation by the International Atherosclerosis Project dealing with the topography of atherosclerotic lesions in the coronary arteries, significant differences were revealed in the 10 to 29 years age group in the severity of atherosclerotic involvement of the right coronary artery between men of Santiago, Chile and New Orleans white males: fatty streak intimal surface involvement (maximal values) 21.2% vs. 47.8%, fibrous plaques (maximal values) 1.0% vs. 10%. Complicated lesions were not recorded in the right coronary artery of the Santiago men and were present in 1% of the New Orleans men.[107]

There are few studies on coronary atherosclerotic involvement of subjects less than 30 years old who died of coronary heart disease. In cases who died in traffic accidents, luminal obstruction was greater than 75%.[146] Autopsied patients with myocardial clinical manifestations showed thrombi in the coronary arteries in 56% of cases and stenosis greater than 75%, and in 44% in one major coronary artery, in 40% in two major coronary arteries, and in 12% in three major vessels.[147] Only 43% of these cases were submitted to the action of the major risk factors for coronary heart disease. In symptomatic coronary heart disease, the 1-year mortality rate for patients aged 21 to 30 years was 3% and 5-year mortality 20%, the cause of death being predominantly cardiac (myocardial infarction, sudden cardiac death, and congestive heart failure).[148] Myocardial revascularization resulted in this age group in a good symptomatic response, a high postoperative activity level, and a reasonable 10-year survival.[149]

Sex differences are also worth mentioning in this age group. The Framingham study found that overt coronary heart disease occurred very rarely in women aged 30 years or less and that myocardial infarction was manifested in only 19% of these patients.[150] A study of the

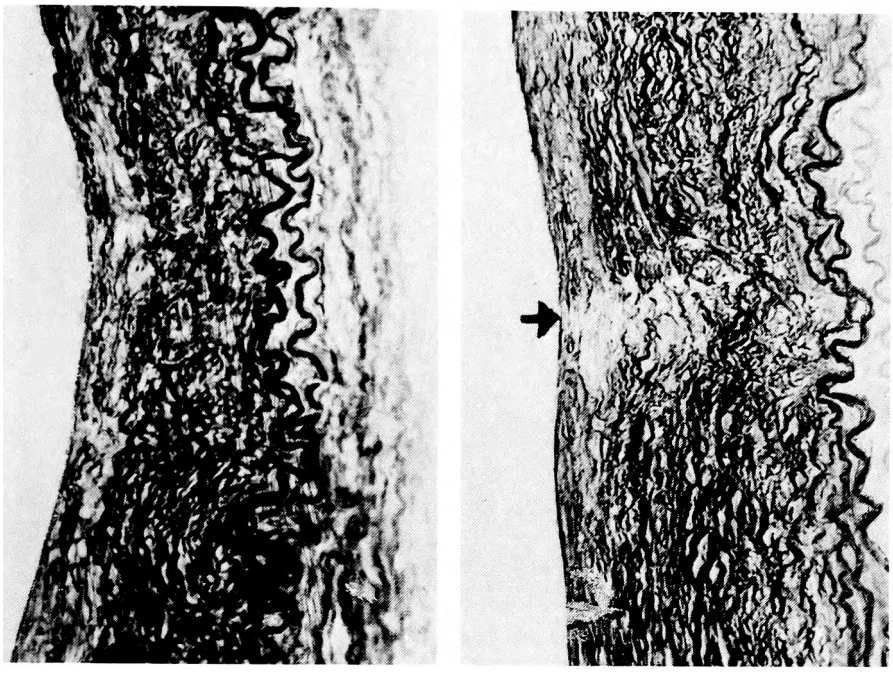


FIGURE 88. Proximal segment of the left anterior descending coronary artery in subjects aged 35 to 40 years. (Left) Subendothelial accumulation of small areas of mucinous material. (Right) Disruptive insudation, extending over the whole intimal layer (arrow) with disintegration of the intimal matrix and progressive occurrence of a gelatinous lesion. (Resorcin fuchsin-alcian blue. Magnification × 220.)

female inhabitants of Göteborg, Sweden revealed that the annual incidence of myocardial infarction in women aged less than 30 years was only 0.03/1000.[151]

IV. ATHEROSCLEROTIC INVOLVEMENT OF THE CORONARY ARTERIES OF ASYMPTOMATIC MATURE ADULTS

A. Mature Adults 31 to 40 Years Old

In these adults investigated in our laboratory, necrosis appeared as the main arterial wall change. There are grounds to suggest that some particular types of intimal necrosis and of plaque necrosis caused lipid to be deposited preferentially in a particular location and in specific forms. Necrosis seemed also to be involved in intramural thrombus formation and in thrombus deposition on preexisting plaques.

For didactic purposes, we have presented coronary artery wall necrosis as developing in three distinct forms visible on light microscopic examination — mucoid, swelling, and dissecting necrosis. We were unable to demonstrate if each of these three forms of necrosis was produced by specific agents and particular pathogenetic mechanisms.

The **mucoid necrosis,** the less severe form, visualized in gelatinous lesions and mucoid plaques, seemed to be produced by a disruptive insudation, able to determine intimal histolysis and replacement of the ground substance and fibers by an edematous-mucinous material (Figure 88). In contradistinction to the mechanical distension produced by simple edema, disruptive insudation led invariably to degradation of the intimal matrix. Only some cells were able to survive and they may acquire the light microscopic feature of lipid-filled cells.

The **swelling necrosis** ("Quellunganekrose", a term coined by German pathologists) is a more severe form of intimal and plaque necrosis, usually visualized on isolated tissue

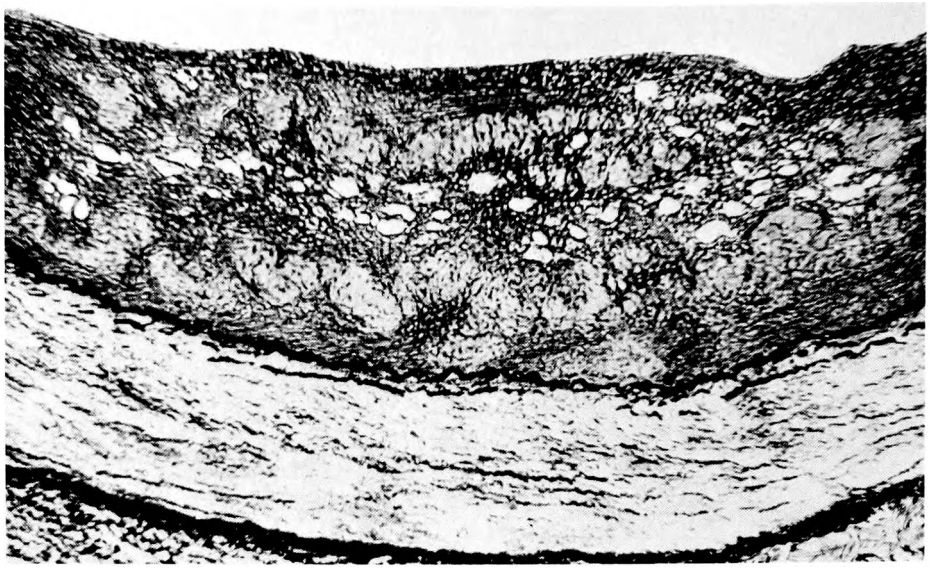


FIGURE 89. Developing necrotic plaque in which only the swelling form of necrosis is present, occurring as globular units intermingled with many foam cells. Of note is the presence of these globular units of swelling necrosis in both the subendothelial and basal intimal regions. (Resorcin fuchsin-alcian blue. Magnification × 220.)

sections as globular units (Figure 89) and/or very thick bands of collagen fibers considerably enlarged in which the microarchitecture was completely lost. The intensity of all histochemical reactions decreased as the aspect turned from mucoid to swelling necrosis. The material included in areas with swelling necrosis persisted after digestion with mucolytic or proteolytic enzymes (sialidase, testicular hyaluronidase, chondroitinase AC and ABC, heparinase, papain, pepsin, trypsin, and collagenase), whereas the material included in areas with mucoid necrosis was rapidly eliminated. The structural change leading to swelling necrosis was associated with diffuse suffusion with tiny lipid droplets in the necrotic mass. On the other hand, swelling necrosis appeared as an inadequate nidus for the accumulation of clusters of foam cells and for the onset of extracellular lipid deposits and cholesterol clefts. An intriguing question is the rarity or even the absence of this form of necrosis in other diseases (tuberculosis, Hodgkin's disease, sarcoidosis, etc.) and in particular immunologic reactions. Whereas the mucoid and dissecting forms of necrosis were frequently seen in many pathological processes, the swelling form appeared as a peculiar change of the arterial wall during the atherosclerotic involvement.

The **dissecting necrosis,** the most severe form, developed as a soft pultaceous debris, usually located in the basal region of the atherosclerotic plaques. This debris included a heterogeneous material rich in fat, fibrin, calcium, disintegrated cells, and fibers, etc., its histochemical reactivity being very complex and ranging from intense to faintly positive for many substances and reactive groups. Particular to the dissecting form of intimal and plaque necrosis was the presence of amorphous lipid deposits and cholesterol clefts.

Intimal necrosis spares the branch sites, being more often visualized in the unbranched segments of the major coronary arteries where it may involve atherosclerotic plaques and all other types of preexisting atherosclerotic lesions, or may develop as an independent lesion (Figure 90) able to give rise to necrotic and fibronecrotic plaques. We found coronary intimal necrosis, occurring as an independent lesion and an early step of atherosclerotic involvement in 6% of children and juveniles, 14% of adolescents, 32% of young adults 21 to 25 years old, and 56% of young adults 26 to 30 years old.[13] This means that before the fourth decade of life, more than $^1/_2$ of the cases investigated showed intimal necrotic areas on light

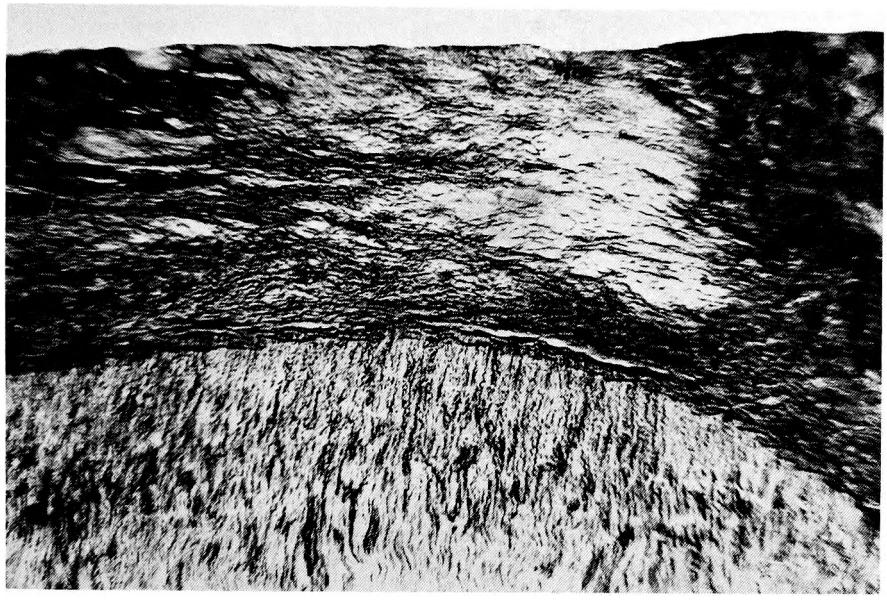


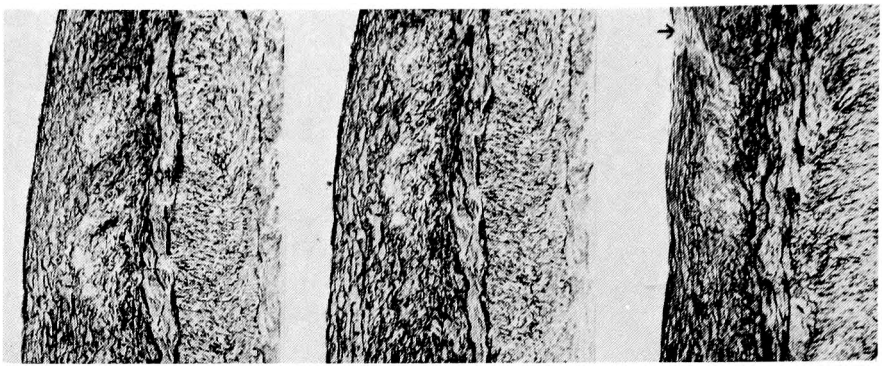
FIGURE 90. Disruptive insudation in a nonbranched area of the intermediate segment of the right coronary artery of a 38-year-old male subject. The light microscopic feature suggests a progressive extension of this necrotizing lesion from the superficial to the basal intimal region. (Resorcin fuchsin-alcian blue. Magnification × 220.)

microscopic examination, whereas on gross inspection all these regions were recorded as normal intima. In subjects aged 31 to 35 years, the proportion of cases with intimal necrosis augmented to 72% and in the 36 to 40 years old group to 84%. The retrospective information on cigarette smoking of deceased subjects revealed more frequent (particularly in heavy smokers) intimal necrotic areas than in nonsmokers of similar age and sex. We also revealed more precocious, extensive, and severe coronary intimal necrosis in systemic lupus erythematosus, dermatomyositis, and sarcoidosis.

Intimal necrosis was frequently associated in our material with the presence of aggregates of inflammatory cells, particularly in the adventitia. A special study carried out on these adventitial inflammatory aggregates revealed their presence in 46% of cases. Both B and T lymphocytes were detected, intermingled with macrophages. These infiltrates seemed to develop as a secondary feature of atherosclerotic involvement.[152]

As a result of the frequent occurrence of necrosis in the coronary arterial wall during the fourth decade of life, a new type of coronary atherosclerotic plaque became prevalent, the fibronecrotic plaque, with a superficial fibrohyaline cap and a basal necrotic center rich in lipid deposits. Both necrotic and fibronecrotic plaques were absent in children and adolescents (except cases with Hurler's syndrome and familial hypercholesterolemia) and represented only 4% of all types of atherosclerotic plaques in young adults 21 to 25 years old. By the end of the third decade of life they accounted for 40% of all coronary atherosclerotic plaques and represented in our material 66% of all plaques in subjects aged 31 to 35 years. During a period of about 15 years, from subjects aged 21 to 35 years, a more than tenfold increase in the proportion of necrotic and fibronecrotic plaques was detected. This dramatic augmentation was associated with a significant diminution in the proportion of mucoid and foam cell-rich plaques. Therefore, in many cases 31 to 40 years old, the histological pattern of coronary atherosclerotic plaques was limited to fibromuscular, necrotic, fibronecrotic, and fibrohyaline plaques. Both fibromuscular and fibrohyaline plaques are usually lipid-free lesions, whereas necrotic and fibronecrotic plaques are lipid-rich.

Serially cut sections and camera lucida drawings allowed us to demonstrate that all necrotic



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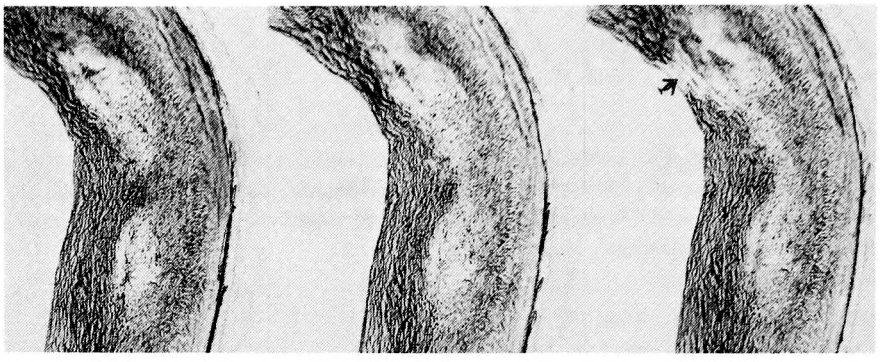


B

FIGURE 91. Serially cut sections showing an intimal necrotic area (A) and necrotic plaque (B) which are connected with the coronary artery lumen (arrow, right) by means of threads of necrotic tissue. They extend like tunnels which perforate the intimal connective tissue. (Resorcin fuchsin-alcian blue. Magnification × 220.)

and fibronecrotic plaques investigated appeared connected with the coronary artery lumen by necrotic bands (Figures 91 and 92). When a necrotic band showed an oblique trajectory or many tortuosities, it occurred on serially cut sections as successive button-like nodules of necrosis. The existence of necrotic and fibronecrotic plaques connected with the coronary arterial lumen by means of necrotic bands was a particular light microscopic feature detected in the coronary arteries of subjects aged 31 to 40 years. It is tempting to think that the necrotic bands appeared first, acting as tunnels through which plasma components rich in necrotizing agents were seeping into the thickened intima and preexisting lesions.

The occurrence of intimal necrosis was mainly related to hypoxia produced by a very thick intima,[145,153] degeneration of intramural thrombi,[31] and degradation of lipid-filled cells as in experimental models submitted to cholesterol-rich diets. According to certain views,[90] the critical determinant for central necrosis and liquefaction in a plaque is the amount of excess lipid and cholesterol it accumulates. In line with this view, only a plaque initially abundant in lipid-cholesterol would be subject to partial dissolution of its internal structure into a greasy paste-like mass, rich in cholesterol and other lipids, and the debris of cells. Our study clearly demonstrated that in human coronary arteries the onset of intimal and plaque necrosis could be related neither to the degree of intimal thickness nor to the presence or abundance of lipid deposits and of lipid-laden cells. We strongly believe that intimal necrosis and plaque necrosis might be considered as an active change produced by some as

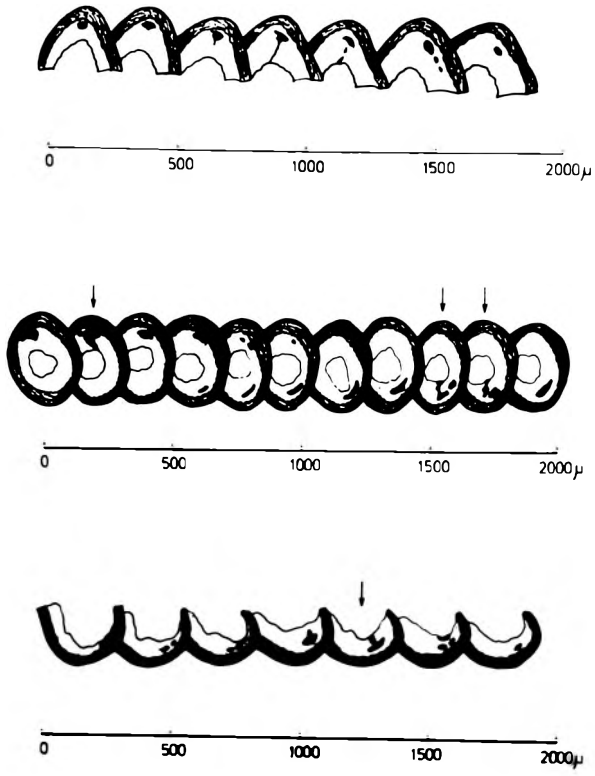


FIGURE 92. Serially cut sections from three coronary arteries (samples removed at 2 cm distal to the point of origin of the anterior descending artery). Male subjects aged 28 (top), 33 (middle), and 34 (bottom) years. Sequential camera lucida drawings to allow for tracing the geometry, diameters, and spatial connections of coronary intimal necrotic areas (in black). Intervening sections have been omitted for clarity. Four intimal necrotic areas which extend centrifugally, in the direction of blood flow, are sketched in black: at the top of the first coronary artery sample; at the top and the bottom of the second coronary artery sample, and at the bottom of the third coronary artery sample. The arrows indicate the connections of intimal necrotic areas with the luminal cavity.

yet unidentified plasma components and associated immune phenomena. These suggestions refer only to our material in which the occurrence of necrosis could not be associated to changes similar to those seen in experimental models. Moreover, as noted before in our material, the atherosclerotic involvement of the coronary arteries progressively acquired the feature of a necrotizing arteriopathy.

By the end of the fourth decade of life, the proportion of cases with atherosclerotic plaques augmented to 66%, with gelatinous lesions and intimal necrotic areas to 84%, with fatty streaks to 82%, and with incorporated microthrombi and intramural thrombi to 22%. This progressive occurrence of new, early atherosclerotic lesions was associated with total serum cholesterol levels of more than 220 mg/dℓ in only 15% of cases, arterial hypertension in more than 10%, and approximately 25% were considered heavy smokers. *The progressive development of new atherosclerotic lesions was associated with an extension of atherosclerotic involvement in the main coronary branch vessels, as well as in the left main coronary artery.*[8] In subjects aged 31 to 35 years, atherosclerotic plaques may develop in the intermediate segment of the left anterior descending and left circumflex arteries, as well as in the proximal segment of the posterior descending, first diagonal, first septal, left marginal, and right marginal vessels. In addition, in subjects aged 36 to 40 years we revealed ather-

osclerotic plaques in the sinus node and atrioventricular node vessels. In essence, during the fourth decade of life, all vessels of the coronary arterial tree with a diameter greater than 1 mm may be involved by atherosclerotic plaques. Plaques continue to develop in the coronary arterial bed during adulthood and such lesions may acquire an advanced form in only 3 to 5 years. This assumption is based on the observation that in successive age groups from 1 to 30 years old we were unable to detect atheroslcerotic plaques, for instance, in the right marginal branch (ramo margus acutus), a vessel used by cardiac surgeons for aortic-coronary bypass grafting. On the other hand, in subjects aged 31 to 35 years, we revealed these plaques in 3% of cases and this indicates that the respective plaques developed during a period of 3 to 5 years without preexistent precursors. Our observations disagree with the largely accepted view that all early lesions appear during childhood and then progress to more advanced forms.

B. Mature Adults 41 to 50 Years Old

1. Pathologic Features of the Major Coronary Arteries

This age group includes men who are particularly at risk to present myocardial clinical manifestations induced by coronary atherosclerosis.

The risk to present myocardial infarction or severe arrhythmias leading to sudden cardiac death involves a subject at a time when he is at his most vigorous, his contributions to society are at a maximum, and his responsibility to his family is the greatest.

Since our study is based on post-mortem examination of apparently healthy individuals dying from a violent cause, one is tempted to presume that the respective population constitutes the best possible cross-sectioned sample for the study of the end stages of the natural history of coronary atherosclerosis. In this population sample, the most particular aspect of the natural history appeared to be the transition from a nodular or patchy atherosclerotic involvement to a diffuse one, associated with a significant increase in the obstructive character of lesions. These changes cannot be revealed by simple gross inspection or by measuring the intimal surface covered with fatty streaks and raised lesions. The transition from a nodular to a diffuse atherosclerotic involvement is mainly due to centrifugal extension of gelatinous lesions, intimal necrotic areas, and necrotic centers of plaques in the direction of the blood flow. This centrifugal extension can be more clearly revealed if the proximal and intermediate segments of the major coronary arteries are cut longitudinally and examined microscopically in a well-established sequence, each sample including 2 to 3 cm of the vessel length. A tridimensional study was also included in this investigation based on serially cut cross-sections and camera lucida drawings. This allowed us to observe and record sequentially, based on these camera lucida drawings, the geometry, diameters, and spatial connections of lesions present in the selected samples (see Figure 92).

Section-to-section tracing of these selected samples revealed uninterrupted intimal necrotic areas or necrotic centers of plaques which could be followed more than 2 cm, the great majority of fibronecrotic plaques investigated by this method showing a longitudinal diameter greater than 1 cm (Figures 93 to 96).

This centrifugal extension could not be demonstrated grossly even if a hand lens was used; we missed the bands interconnecting large intimal necrotic areas and, in particular, we missed the great majority of these intimal necrotic areas. The fibronecrotic plaques interconnected by thin or thick necrotic bands were erroneously grossly recorded by us as individual lesions. On the photographic reconstruction of the respective longitudinally cut sample they appeared only as sites exhibiting more advanced pathologic changes in a very long, uninterrupted atherosclerotic involvement. In the presence of this diffuse atherosclerotic involvement, all attempts to record the intimal surface covered with raised lesions may offer data only at the tip of the iceberg, whereas its base remains undetected and is considered normal intima.

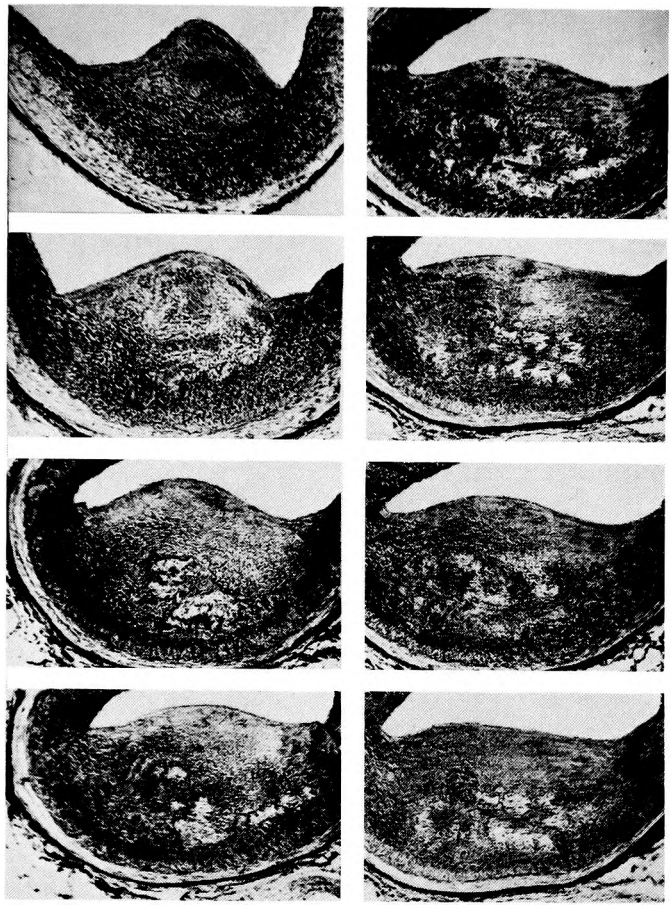


FIGURE 93. Eight serial cross-sections along the epicardial course of the proximal segment of the left anterior descending coronary artery of a 49-year-old woman who died in a traffic accident. (Left) From top to bottom and (right) from top to bottom, each micrograph is separated from the next by 0.05 cm. These apparently different histologic types of lesions belong to an uninterrupted fibronecrotic plaque with a necrotic center which extends more than 1 cm along the longitudinal vessel axis. (Resorcin fuchsin-alcian blue. Magnification × 40.)

Our own experience demonstrates that the tridimensional study is the best to demonstrate the diffuse character of coronary atherosclerotic involvement in subjects aged 41 to 50 years. On the other hand, this method is difficult to perform, requiring the visualization of thousands of stained tissue sections and the superposition of hundreds of camera lucida drawings. For these reasons, it could not be extended to a more important number of cases and selected samples.

To overcome this difficulty, we used an additional simple and rapid technique: successive longitudinal samples. At a × 20 magnification, 8 to 12 successive microphotographs grouped in sequence were necessary to reconstruct each longitudinal sample (see Figures 94 to 96).

The centrifugal extension of many intimal necrotic areas and necrotic and fibronecrotic plaques was associated with the existence of a particular microarchitecture at the distal side of the lesion, namely at the point where the respective lesions irradiated centrifugally in the direction of blood flow. This distal side occurred as a more irregular border, by far more irregular than the proximal, lateral, luminal, and medial sides of the same very long lesion. Serial reconstruction of successive cross-sections revealed a highly irregular boundary with numerous peninsulas of interdigitating necrotic and apparently normal intimal connective

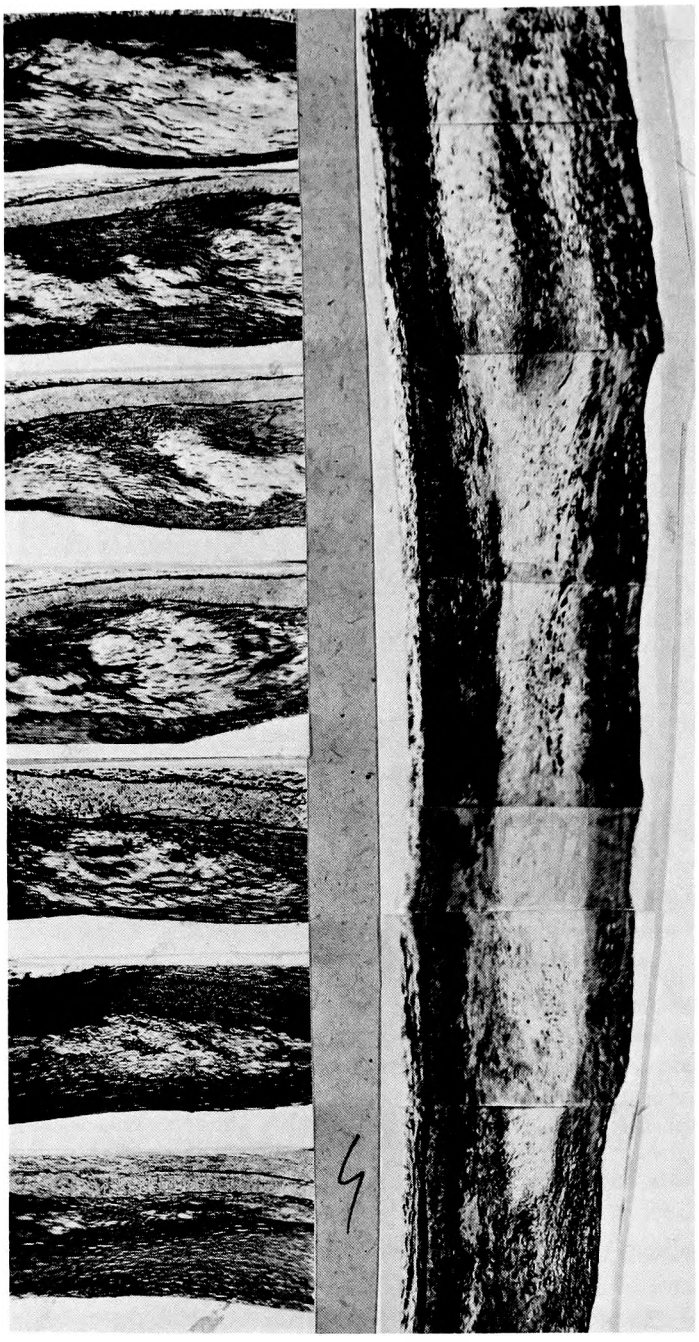


FIGURE 94. (Left) Seven separate micrographs from a longitudinally cut sample removed from the proximal segment of the left anterior descending coronary artery of a 49-year-old male subject dead in a car accident. Each micrograph has its own light microscopic feature, suggesting seven independent lesions occurring as intimal necrotic areas, necrotic plaques, and fibronecrotic plaques. (Right) Seven micrographs from a longitudinal sample removed from the proximal segment of the left anterior descending coronary artery of a 49-year-old male subject dead in a traffic accident. The micrographs were put in sequence (from top to bottom) in the direction of blood flow, a photographic reconstruction of the whole longitudinal sample thus being obtained. The diffuse extension of the fibronecrotic plaque is clearly demonstrated by the latter method. (Resorcin fuchsin-alcian blue. Magnification × 20.)

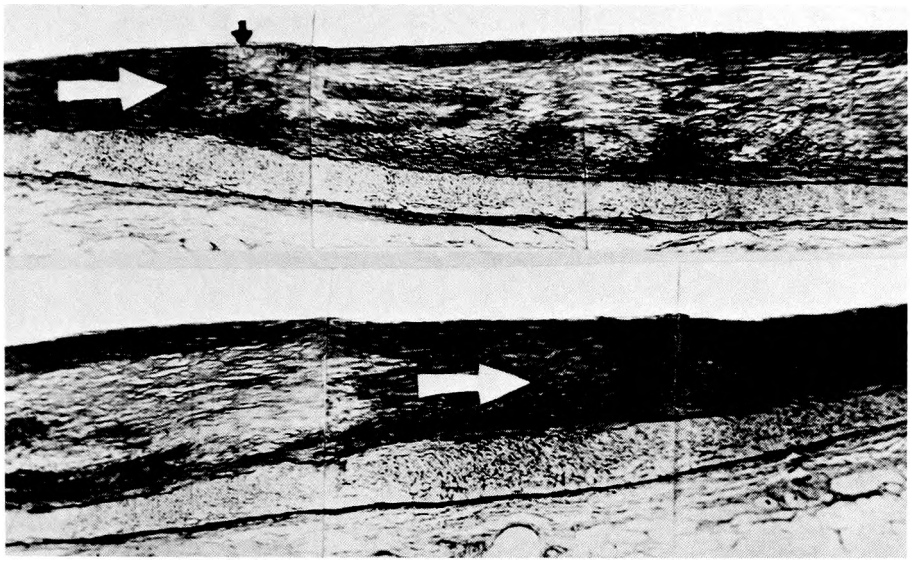


FIGURE 95. A longitudinally cut sample removed from the proximal segment of the right coronary artery of a 50-year-old woman dead in a traffic accident. It includes parts of centimeters 2 and 3 of the epicardial course of the right coronary artery, the total length of the sample removed equal to 1.6 cm. A diffuse centrifugal extension of coronary atherosclerotic involvement can be observed, occurring as interconnected intimal necrotic areas, necrotic plaques, and fibronecrotic plaques. The white arrows indicate the direction of blood flow; the black arrow indicates a region where a massive insudation gave rise to a gelatinous lesion, then to an intimal necrotic area which extended in the direction of blood flow, involving more than 1.0 cm of the vessel course. (Resorcin fuchsin-alcian blue. Magnification × 20.)

tissue. This highly irregular boundary might appear as a border zone and might be considered as a region of intermediate injury between a gelatinous lesion, an intimal necrotic area, or a necrotic center of a plaque on the one hand, and apparently unaltered intimal connective tissue, on the other. This was clearly seen on longitudinally cut samples, since the light microscopic examination of stained tissue sections revealed the existence of three zones: an area of intimal necrosis, an area of intermediate injury, exhibiting aspects of progressive intimal histolysis, and an area of apparently normal intimal connective tissue.

The area of intermediate injury or border zone occurred as a region of 50 to 100 μm where a wavefront of necrosis began to produce irreversible alterations of the intimal matrix in the direction of the blood flow (Figure 97). The border zone did not appear in our material as a histologic entity, but only as a time-dependent pathological change; it seemed to shrink more rapidly than the other sides of the lesions and therefore it might be considered as an intimal region which is jeopardized or at risk.

Our results emphasized the centrifugal extension of some coronary gelatinous lesions, intimal necrotic areas, and necrotic centers of plaques as an important event in the natural history of coronary atherosclerosis in subjects aged 41 to 50 years. This extension in the direction of the blood flow gives rise to the onset of very long lesions and to the aspect of diffuse atherosclerotic involvement.

The various stages of the centrifugal extension of lesions seemed to depend on the microarchitectural characteristics of the intimal connective tissue and of its response capabilities. Also of particular importance appeared to be the dissecting and necrotizing power of some as yet unidentified agents present in the necrotic material. The rapidity with which the border zone of intermediate injury is disintegrated and the degree of interdigitation between normal and necrotic intimal connective tissue reflect the histolytic capacity of these highly potent glycolytic and proteolytic agents, which are able to disintegrate very thick collagen bundles

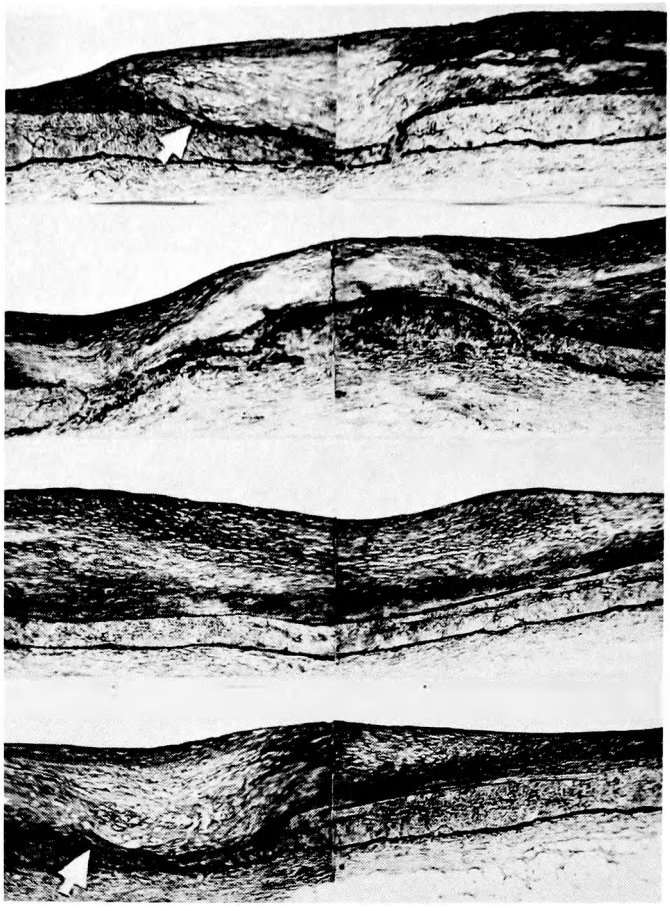


FIGURE 96. A longitudinally cut sample removed from the proximal segment of the right coronary artery of a 52-year-old woman dead in a traffic accident. It includes parts of centimeters 3 and 4 of the epicardial course of the right coronary artery, the total length of the sample removed being 1.6 cm. A diffuse centrifugal extension of coronary atherosclerotic involvement can be observed, occurring as interconnected intimal necrotic areas, necrotic plaques, and fibronecrotic plaques. The two fibronecrotic plaques (arrows) appear at the beginning and at the end of this very long lesion. (Resorcin fuchsin-alcian blue. Magnification × 20.)

and elastic laminae and even hyalinized fibrous intima (Figure 98). It is tempting to assume that the presence or absence of a border zone located between necrotic and normal intimal connective tissue may partly account for the aggressive or stabilized character of certain gelatinous lesions, intimal necrotic areas, and necrotic centers of plaques. The spatial geometry of an aggressive lesion seems to imply the existence of a functional border zone. Similar assumptions can be made concerning the extension of lesions toward the luminal area and this may be followed by plaque rupture, ulceration, and occurrence of occlusive thrombosis.

The local and general conditions which induce a rapid or a slow wavefront of necrosis at the distal side of some atherosclerotic plaques are not clear. The extension along the longitudinal axis of the major coronary arteries follows the previous extension of the thickened intima (Figure 99). This would mean that a diffuse intimal thickening appears as a prerequisite for the centrifugal extension of lesions. In addition, associated diseases seem to influence the onset of very long lesions and the diffuse character of coronary atherosclerotic involve-

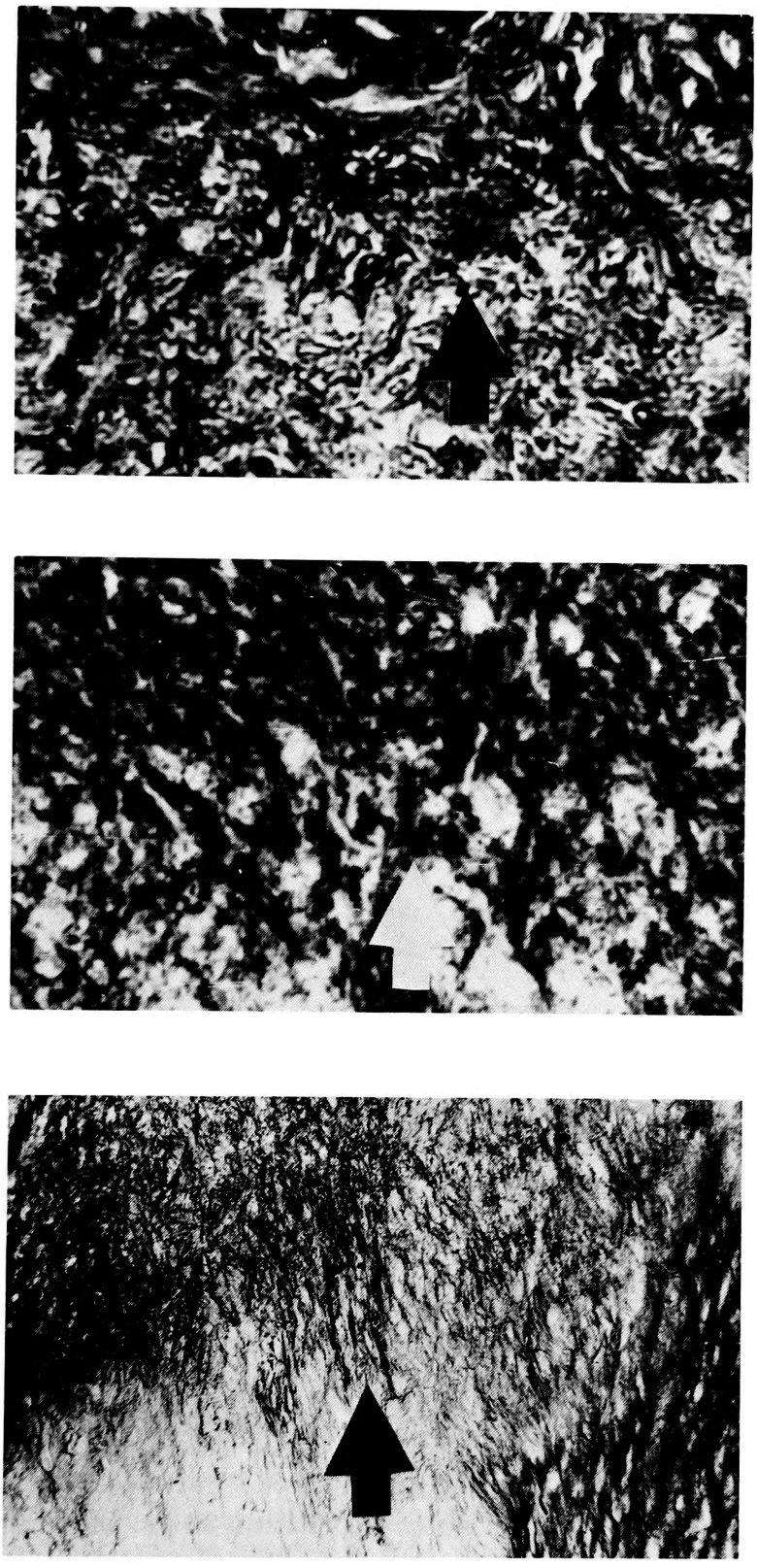
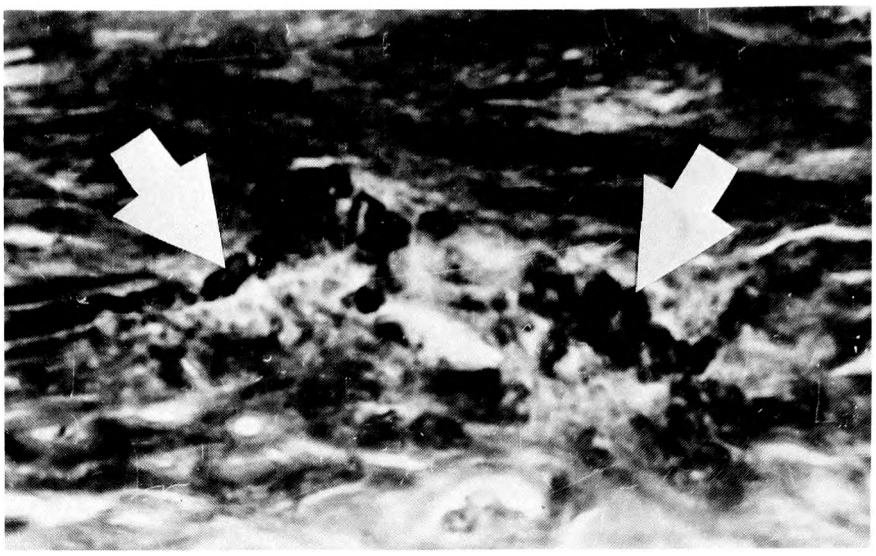


FIGURE 97. Three aspects of progressive intimal histolysis occurring as a border zone (arrows) of intermediate injury, interposed between necrotic mass and apparently normal connective tissue. (A) Degradation of the ground substance and cells (toluidine blue staining); (B) degradation of the argyrophilic networks (Gömöri's silver impregnation); (C) degradation of fuchsinophilic fibers (Van Gieson's picrofuchsin). (Magnification × 748.)



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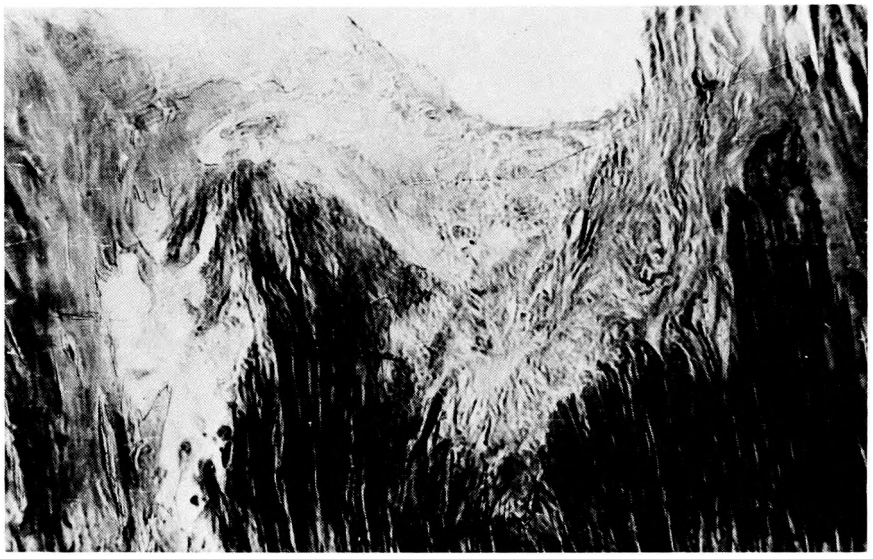


B

FIGURE 98. The histolytic capacity of the necrotic areas which extend and produce a diffuse atherosclerotic involvement in subjects aged 41 to 50 years is sometimes considerable. The agents present in the necrotic mass are able to disintegrate thick elastic laminae (A, arrows) and even hyalinized fibrous intimas (B). (A, orcein, picrofuchsin and B, Van Gieson's picrofuchsin. Magnification × 880.)

ment in mature adults. This diffuse character was constantly detected in patients with systemic lupus erythematosus and with other diseases with important immunologic injuries.

2. Pathologic Features of the Main Branch Vessels

Many pathologists consider that the atherosclerotic involvement of human coronary arteries is limited to major vessels: left main coronary artery, left anterior descending artery, left

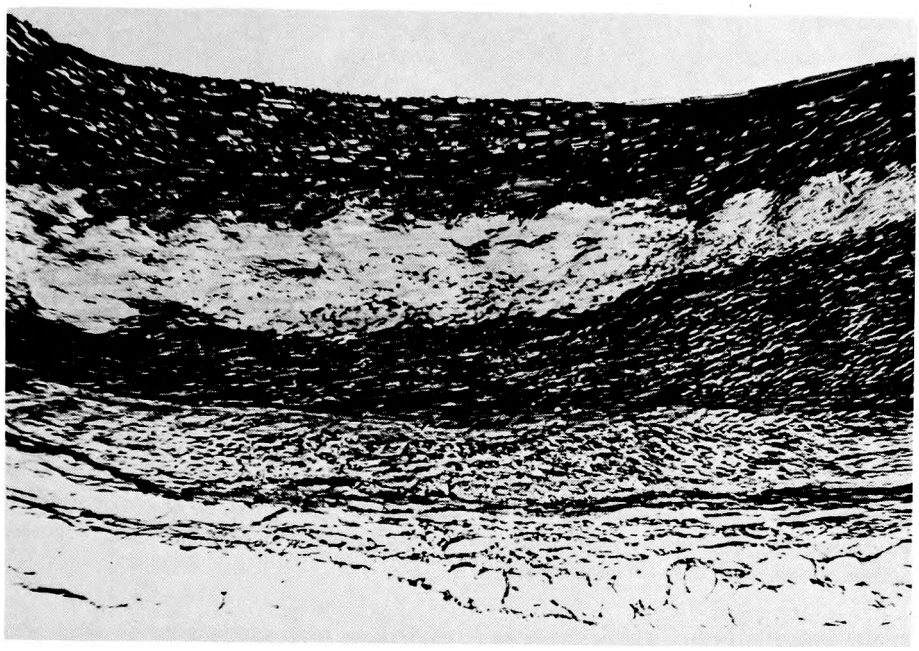


FIGURE 99. The extension of areas of necrosis requires as a prerequisite the presence of a very thick intima which forms the nidus for this extension. On in vivo angiographic examinations, this vessel segment appeared without disease and on post-mortem examination, it was grossly recorded as normal intima, since it did not include fatty streaks or raised lesions. (Resorcin fuchsin-alcian blue. Magnification × 220.)

circumflex artery, and right coronary artery. In fact, only these major vessels are usually longitudinally opened during routine autopsy.

Many pathologists also accept a particular involvement of the small intramyocardial vessels. This pathology is considered nonatherosclerotic in character and includes inflammatory, immune, metabolic, degenerative, and hereditary lesions associated with platelet aggregates and microemboli or microthrombi. The complex light-microscopic feature of these pathologic changes is termed "small vessel disease".[154-155]

As concerns the atherosclerotic involvement of coronary branch vessels with an external diameter of 1 to 3 mm (sometimes similar to that of a major coronary artery), we were unable to find special studies in the available literature. This is probably due to methodologic limitations of autopsy protocols which overlook the presence of atherosclerotic lesions in coronary branch vessels. Certain standard operating protocols even recommend "to exclude from the study all branches".[156] A strict adherence to these protocols neglects the possible presence of stenotic or occlusive atherosclerotic plaques in the posterior descending, first diagonal, first septal, left marginal, right marginal, sinus node, and atrioventricular node vessels. Such methodologic limitations contrast with the growing interest of cardiac surgeons and angiographers in the identification of stenotic or occlusive atherosclerotic plaques in coronary branch vessels. This interest can be related to the external diameter of these branches which may equal that of a major coronary artery and to their epicardial course adequate for grafting. In many cases, cardiac surgeons prefer to graft into the posterior descending or the first diagonal, or a marginal vessel, than into a narrowed intermediate segment of the right coronary artery, left anterior descending artery, or left circumflex artery.

In a study on the significance of coronary arterial thrombosis in transmural acute myocardial infarction, Brosius and Roberts[46] present patients in whom the thrombus existed only in the posterior descending branch of the right coronary artery, or only in the left marginal

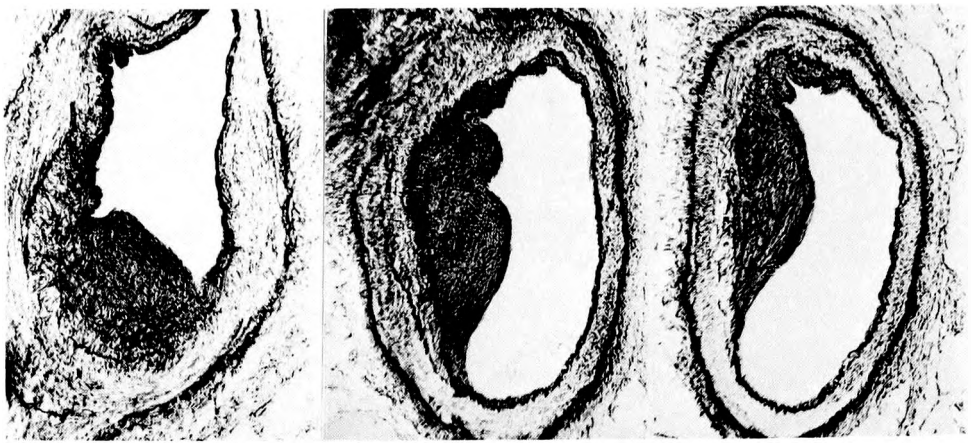


FIGURE 100. The proximal segment of the posterior descending branch of the right coronary artery in 3 subjects aged 35 (left), 38 (middle), and 39 (right) years. Occurrence of fibromuscular plaques without a preexisting thickened intima or other type of precursor. (From Velican, C. and Velican, D., *Atherosclerosis*, 60, 237, 1986. With permission.)

branch of the left circumflex artery. It is easy to deduce how unrealistic an anatomoclinical correlation would be in such cases if the respective branches were excluded from the study.

A severe degree of atherosclerotic involvement was revealed in the sinus node and atrioventricular node branches in the hearts of patients who died of severe arrhythmias followed by sudden cardiac death; this could be correlated not only with the clinical events, but also with a fibrosis of the conducting tissue.[157] These vessels which supply the conduction system are neglected even in anatomoclinical correlations related to patients with severe electrical disorders of the heart rhythm, followed by sudden cardiac death.

In a recent investigation of our laboratory[16] on the atherosclerotic involvement of coronary branch vessels (posterior descending, first diagonal, first septal, left marginal, right marginal, sinus node, and atrioventricular node vessels), we found that *one out of every three persons with atherosclerotic plaques in the major coronary arteries also had plaques in coronary branch vessels.*

In our material, likewise, one of every three subjects had more than 50% lumen reduction in the undistended major coronary arteries, compared to one out of every six cases in undistended branch vessels. Moreover, we could delineate a small group, including 8% of the subjects investigated with a net prevalence of women, which showed more severe atherosclerotic plaques in the coronary branch vessels than in the major coronary arteries. Some of these stenotic plaques developed in the absence of a preexisting thickened intima (Figure 100).

In the 41 to 50 years age group in the major coronary arteries compared to the main branch vessels there were

- 75% subjects vs. 28% subjects with atherosclerotic plaques
- 86% subjects vs. 6% subjects with fatty streaks
- 97% subjects vs. 12% subjects with gelatinous lesions and intimal necrotic areas
- 34% subjects vs. 14% subjects with incorporated microthrombi and intramural thrombi

This clearly demonstrates that the pathology of the main coronary branch vessels as related to atherosclerotic involvement has a particular feature: a strong prevalence of atherosclerotic plaques over other types of lesions (Table 16).[16]

Likewise, in subjects aged 41 to 50 years, in the major coronary arteries compared to the main branch vessels there were

Table 16
PERCENT OF SUBJECTS WITH ATHEROSCLEROTIC PLAQUES, FATTY STREAKS, INTIMAL NECROTIC AREAS, AND INCORPORATED MICROTHROMBI IN THE MAJOR CORONARY ARTERIES (MCA) AND IN THE LARGE- AND MEDIUM-SIZED CORONARY BRANCH VESSELS (cbv)

Age groups (years)	Subjects (%) with atherosclerotic lesions											
	Plaques			Fatty streaks			Intimal necrotic areas			Incorporated microthrombi		
	MCA	cbv	Ratio	MCA	cbv	Ratio	MCA	cbv	Ratio	MCA	cbv	Ratio
11—15	4	0		6	0		6	0		3	0	
16—20	12	0		34	0		13	0		8	0	
21—25	28	4	7.0	52	2	26.0	32	0		10	0	
26—30	42	10	4.2	66	2	33.0	56	2	28.0	14	0	
31—35	58	18	3.2	66	3	22.0	72	8	9.0	18	0	
36—40	66	20	3.3	84	4	22.0	84	10	8.4	22	0	
41—45	72	26	2.7	80	6	13.3	96	10	9.6	32	2	16.0
46—50	88	30	2.9	72	6	12.0	98	14	7.0	36	6	6.0
51—55	90	32	2.8	68	6	11.3	98	14	7.0	44	6	7.3

From Velican, C. and Velican, D., *Atherosclerosis*, 60, 237, 1986. With permission.

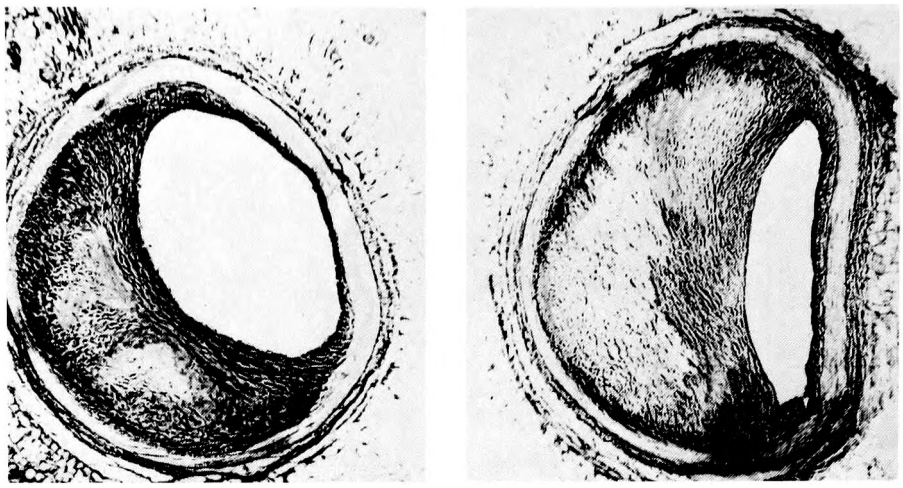


FIGURE 101. The proximal segment of the posterior descending branch of the right coronary artery in two subjects aged 46 (left) and 50 (right) years. Progression of fibromuscular plaques to typical fibronecrotic plaques. (Resorcin fuchsin-alcian blue. Magnification × 220.)

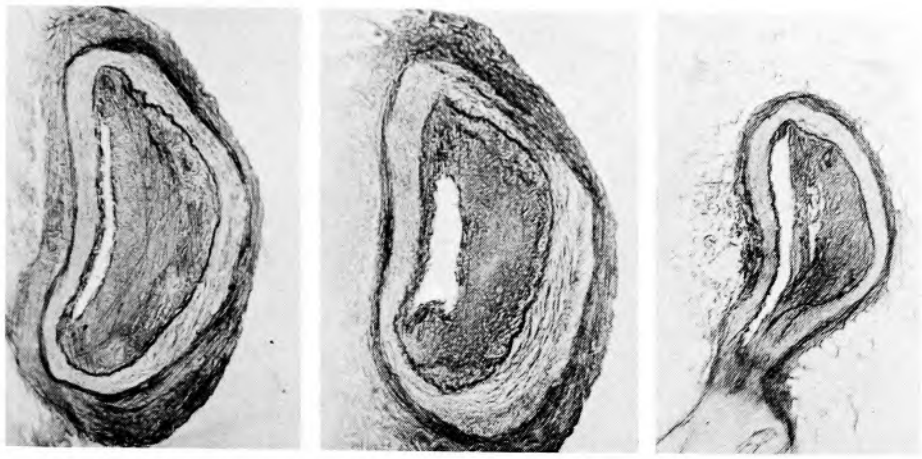
- 30 vs. 10 fibromuscular plaques
- 16 vs. 6 necrotic plaques
- 38 vs. 14 fibronecrotic plaques
- 40 vs. 12 fibrohyaline plaques

The plaques present in the proximal segment of the main branch vessels did not show an adventitia-derived network of blood capillaries, or capillaries derived from the vessel lumen, ulcerations, hemorrhage, calcifications, cracks, or breaks. In our material, the most severe plaques developed in coronary branch vessels were seen in the proximal segment of the posterior descending (Figure 101) and first diagonal arteries and certain of these plaques might acquire the features of a complicated lesion as in the major coronary arteries.

On the other hand, as the external diameter of the branch vessel decreased, the plaques exhibited an underdeveloped character, including a more compact, nonhyalinized connective tissue in its subendothelial region and small areas of mucoid necrosis, usually lipid-free, in its basal region. These plaques might be considered intermediate stages between a gelatinous lesion and an advanced fibrohyaline plaque. In branch vessels with an external diameter of about 1 mm, the prevalent or even unique type of plaque was the fibromuscular plaque, which blended with very thick intimas.

The prevalent light microscopic feature of both well-developed and apparently underdeveloped atherosclerotic plaques appeared the stenotic character of the lesions in their tendency to encroach upon the vessel lumen (Figures 102 and 103). Whereas in the major coronary arteries this tendency could be related to superposition of microthrombi on preexisting stenotic plaques, abundant accumulation of lipids in the necrotic center of the plaque, or intraplaque hemorrhage, in the main coronary branch vessels only an increase in the lesion size was associated with its obstructive character. More than 75% luminal reduction in undistended major coronary arteries was revealed in subjects aged 41 to 50 years in 24% of the cases, and more than 75% luminal reduction in undistended main branch vessels was detected in the same sample in 12% of cases.

In essence, a pathology of the main coronary branch vessels exists related to the natural history of coronary atherosclerosis, its importance in certain patients being considerable. This pathology is usually neglected due to a strict adherence to available protocols, overlooked even in studies dealing specifically with coronary atherosclerosis and coronary heart



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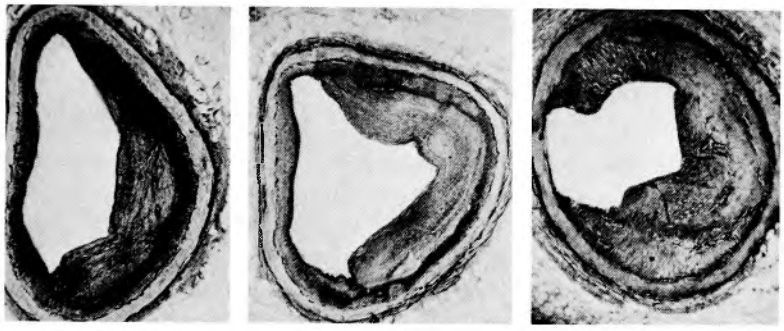

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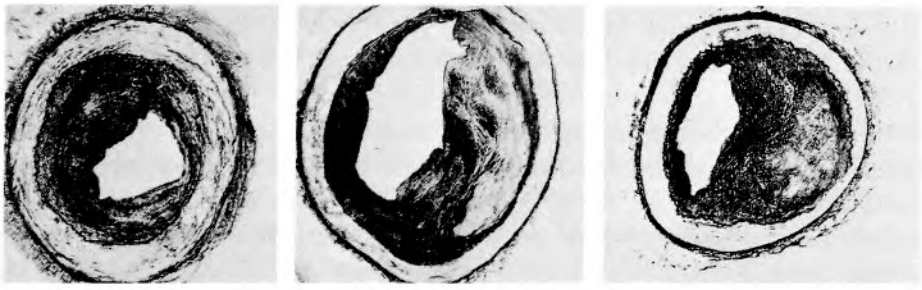

C

FIGURE 102. (A) Grossly neglected obstructive lesions, blocking more than 75% of the lumen of the first septal branch. (B) A grossly neglected lesion blocking more than 70% of the lumen of the right marginal branch. (C) A grossly neglected lesion blocking more than 80% of the lumen of the left marginal branch. (From Velican, C. and Velican, D., *Atherosclerosis*, 47, 215, 1983. With permission.)

disease. This might lead to erroneous clinico-pathological correlations and to unrealistic assumptions on the existence of myocardial clinical manifestations without severe coronary atherosclerotic involvement. In many cases of myocardial infarction or sudden cardiac death without occlusive lesions in the proximal segment of the major coronary arteries, the ulterior investigation of the main branch vessels revealed such occlusive lesions in the posterior

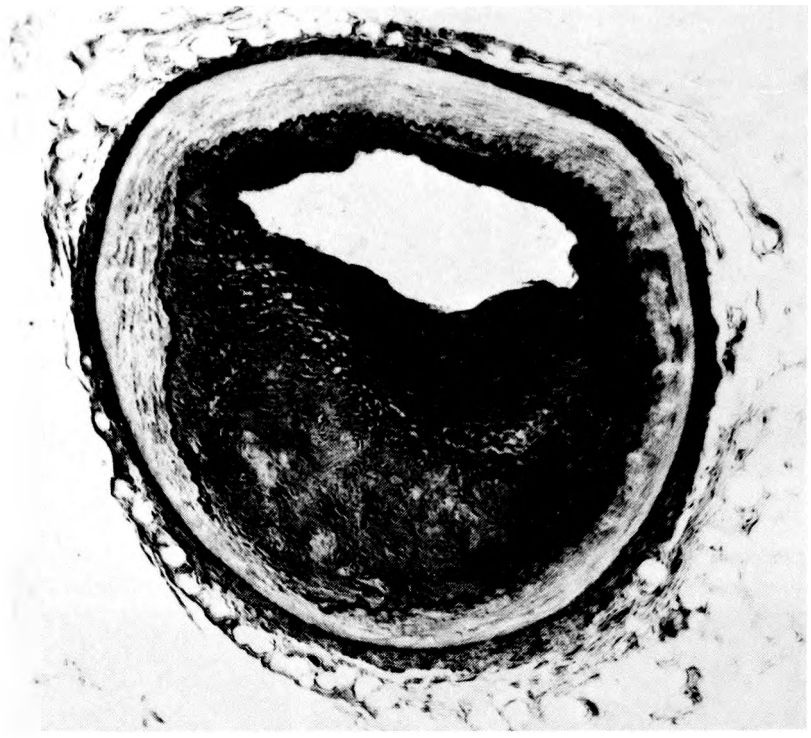

FIGURE 103. A typical plaque found in the proximal segment of the main coronary branch vessels. It lacks a large necrotic center, abundant lipid deposition, a thick fibrous cap, and a superimposed thrombus; on the other hand, it exhibits an important stenotic character. Proximal segment of the first diagonal branch of a 52-year-old man who died of acute anterolateral myocardial infarction. (Resorcin fuchsin-alcian blue. Magnification × 80.)

descending artery, first septal artery, or atrioventricular node artery. The possible involvement of stenotic or occlusive lesions developed in the coronary main branch vessels in the pathogenetic lesions developed in the coronary main branch vessels in the pathogenetic mechanisms of coronary heart disease was repeatedly suggested and convincingly demonstrated.[154,155,157-163]

In addition to the above-mentioned possible clinical significance, the discovery of severe narrowings in the coronary branch vessels may help the pathologist to surpass a difficult moment, when he must assume, based on the negative results of routine gross inspection, that the patient showed nothing that he could define as cause of myocardial infarction or of sudden cardiac death.

Unrecognized occlusive plaques may lead to errors in clinico-pathologic correlations and Figure 104 is very demonstrative in this respect. There were several cases in our material with severe stenotic plaques in coronary main branch vessels associated with a lack of severe atherosclerotic involvement in the segments of the major coronary arteries which are usually opened during routine autopsy. It might be also of interest to call that if in 100 male adults aged 51 to 55 years only routine gross inspection was performed, we revealed 52% cases with luminal narrowing of more than 50% in major coronary arteries. If gross inspection was extended to the proximal segment of the posterior descending, first diagonal, first septal, and marginal vessels, then the proportion of subjects with luminal narrowing of more than 50% in the same sample augmented to 61%. Finally, if gross inspection was associated with light microscopy, including the distal segments of the major coronary arteries and branch vessels up to 1 mm in external diameter, the proportion of subjects with luminal narrowing more than 50% augmented again, in the same sample, from 61 to 73%.

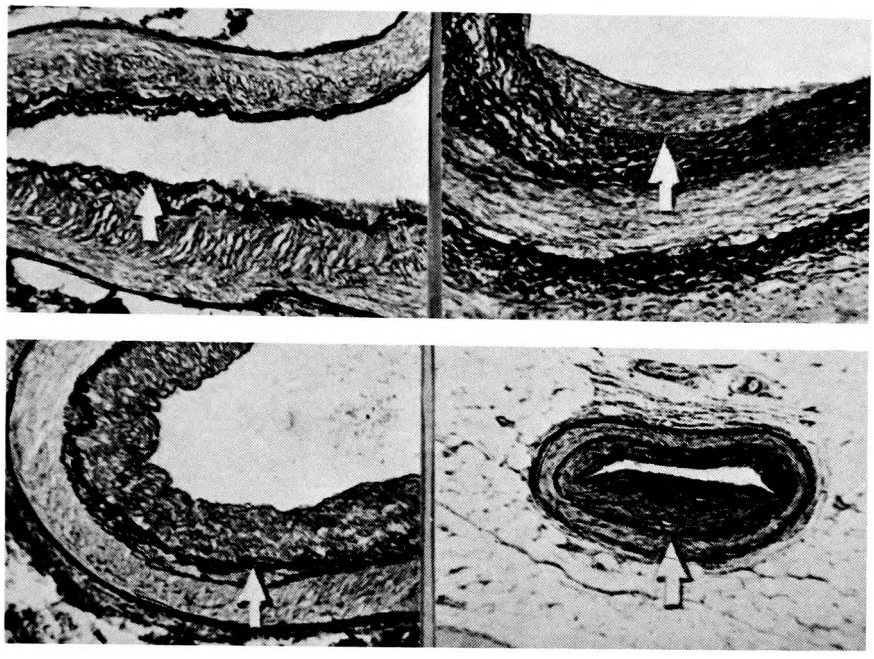

FIGURE 104. A 45-year-old female subject showing a very thin intima in the proximal segment of the right coronary artery (top left, arrow) an intima as thick as the underlying media in the proximal segment of the left circumflex artery (bottom left, arrow) and a thick, stratified, intimal layer in the proximal segment of the left anterior descending artery (top, right arrow).The absence of atherosclerotic plaques in the major coronary arteries contrasted with the presence of a stenotic plaque in the proximal segment of the first diagonal branch (bottom, right arrow). (From Velican, C. and Velican, D., *Atherosclerosis*, 47, 215, 1983. With permission.)

3. Development of Lesions of Possible Clinical Significance

Starting from the complex pathology related to atherosclerotic involvement of the major coronary arteries and the main branch vessels, an attempt has been made to obtain a dynamic view of the natural history of coronary atherosclerotic lesions. In our material, the main pathways of progression from early to advanced lesions is represented by the passage from fibromuscular to fibrohyaline and fibronecrotic plaques.

Additional pathways are represented by the passage from gelatinous lesions and small intimal necrotic areas to necrotic, fibronecrotic, or fibrohyaline plaques; by the formation of fibrohyaline plaques as a result of successive microthrombi encrustation and/or direct intramural thrombus formation; by the passage from fibromuscular to fibronecrotic plaques having as intermediate stages the mucoid and foam cell-rich plaques; and by the passage from advanced fatty streaks to fibronecrotic plaques. This complex evolution is presented in Figure 105, which suggests three main pathogenetic mechanisms involved in atherogenesis: abnormal permeation, abnormal proliferation, and abnormal coagulation. This figure tries to arbitrarily delineate early, intermediate, and advanced lesions and suggests how the early lesions might be related temporally to intermediate and advanced lesion. Figure 106 refers especially to the suggested cycle of evolution of fibromuscular plaques.

The age-related increase in the percentage of subjects with atherosclerotic plaques, gelatinous lesions and intimal necrotic areas, fatty streaks, incorporated thrombi, and intramural thrombi, from childhood up to the age of 50, is presented diagramatically in Figures 107 to 110. In these figures, special emphasis is placed on suggested preexisting and newly formed lesions in each age group. The number of preexisting cases with atherosclerotic plaques or other types of lesion was considered that of the preceding age group; the number

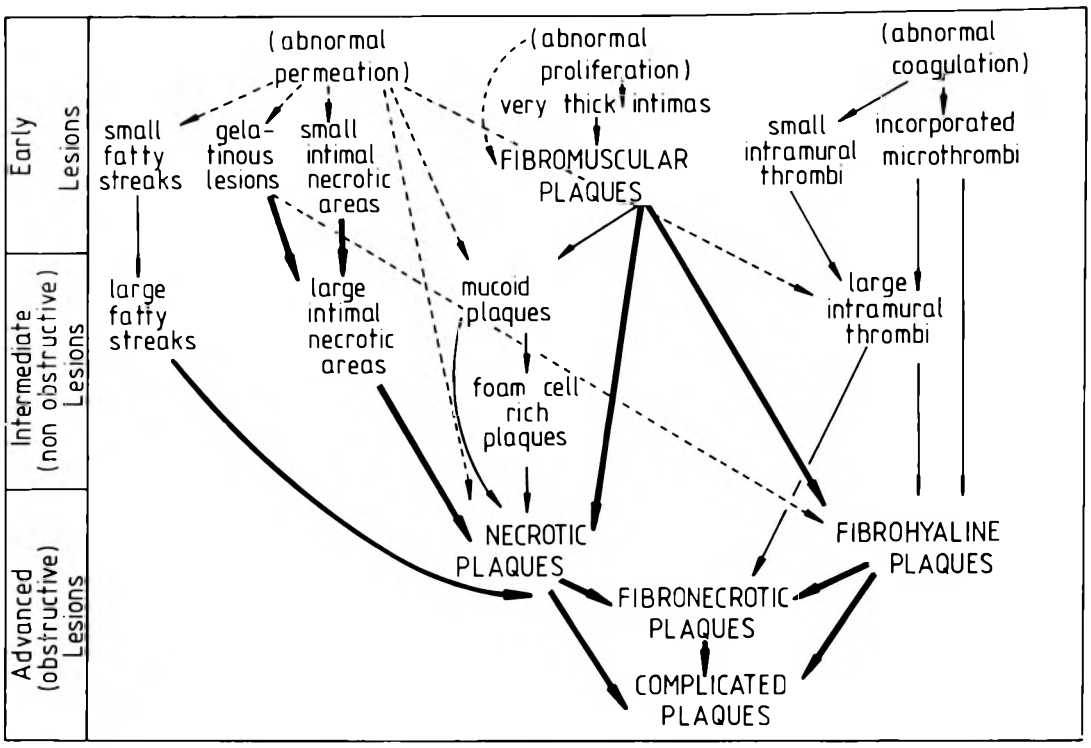

FIGURE 105. Suggested progression of coronary atherosclerotic lesions as revealed by our studies. Abnormal permeation, abnormal proliferation, and abnormal coagulation appear as the most important precipitating causes in the onset of lesions and their ulterior progression toward advanced plaques. The thick solid lines indicate the main pathways of progression from early to advanced lesions; the thin solid lines, the secondary pathways; and the dotted lines, some pathogenetic mechanisms involved.

of newly formed lesions was deduced by subtracting the number of positive cases of the preceding age group from the number of positive cases of the succeeding age group. Figures 107 to 110 show that in our unselected Bucharest population sample, the oldest (46 to 50 years) age group exhibited 100% gelatinous lesions and intimal necrotic areas in their coronary arteries, 96% various histologic types of atherosclerotic plaques, 83% fatty streaks, and 48% microthrombi and intramural thrombi. During a period of life of about 10 years, from the 36 to 40 years age group to the 46 to 50 years age group, the number of subjects with atherosclerotic plaques increased 1.33 times, with gelatinous lesions and intimal necrotic areas 1.19 times, with incorporated microthrombi and intramural thrombi 2.18 times, and with fatty streaks 1.01 times. These results demonstrate that atherosclerotic involvement of the coronary arteries of subjects aged 41 to 50 years appeared dominated by an *important occurrence of microthrombi, juxtaposed on preexisting and newly formed atherosclerotic plaques* (Figure 111).

The ratio of fibrohyaline or fibronecrotic plaques with vs. fibrohyaline or fibronecrotic plaques without incorporated microthrombi was 0.80 in subjects aged 31 to 35 years and 1.57 in subjects aged 41 to 45 years.[17] *This age-related increase in lesions with incorporated microthrombi ran parallel to an age-related increase in luminal narrowing.* Also of note is our observation that the number of plaques with incorporated microthrombi increased up to 3.6 times from one 5-year age group to the next in heavy cigarette smokers and only up to 1.4 times in nonsmokers of similar age and sex, the difference being statistically significant.

The evolution of some microthrombi toward advanced lesions was characterized by successive phases of encrustation, associated with an avascular organization of intramural fibrin networks, leading to the onset of stenotic fibrohyaline plaques. The plaques on which microthrombi were deposited usually lost their affinity for many stains and showed a rapid

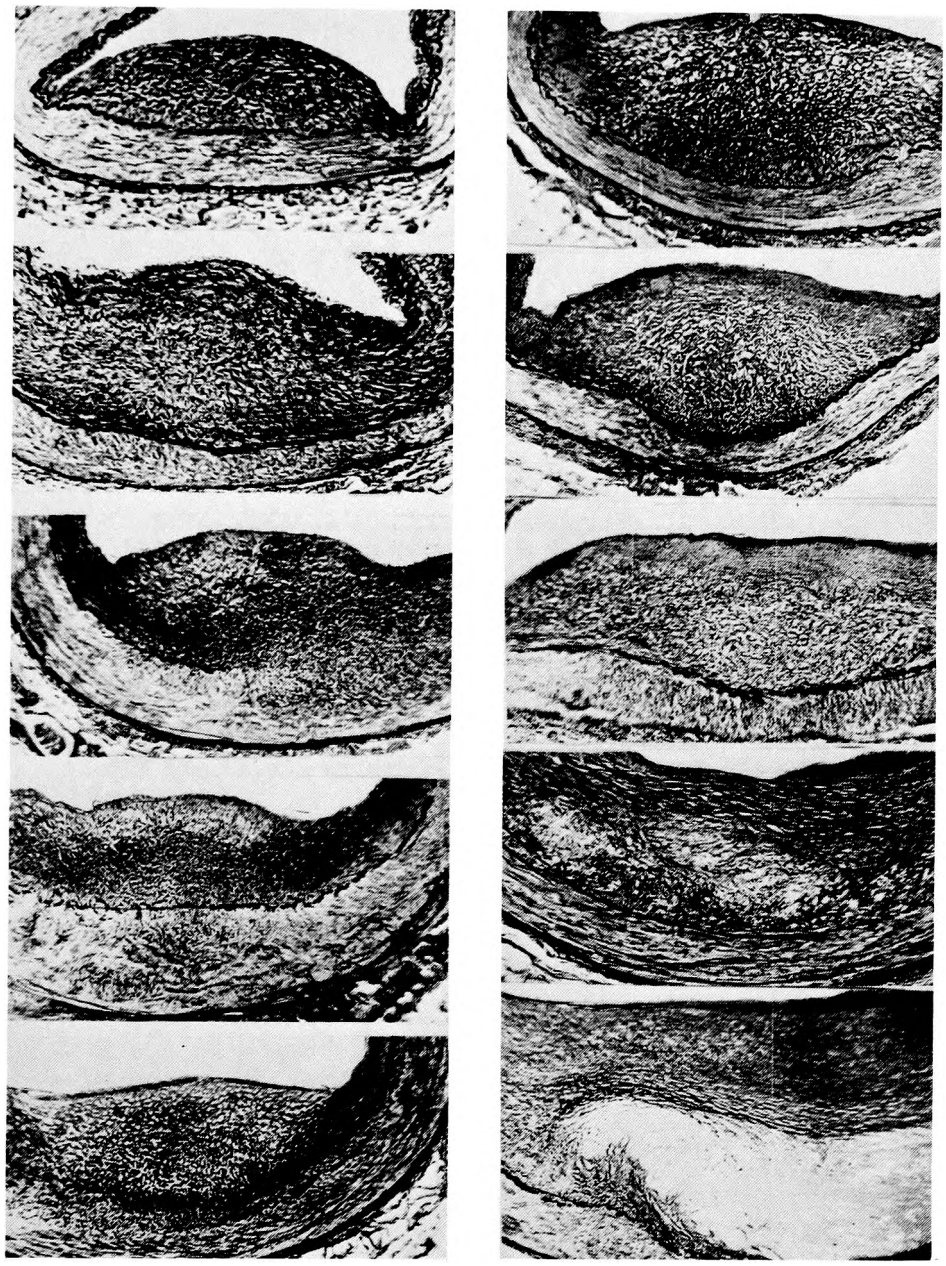

FIGURE 106. Light microscopic features of various histologic types of coronary atherosclerotic plaques, placed in sequence against age. (Left, from top to bottom) Two fibromuscular plaques, two mucoid plaques, and a foam cell-rich plaque. (Right, from top to bottom) A foam cell-rich plaque, two necrotic plaques, two fibronecrotic plaques. (From Velican, C. and Velican, D., *Atherosclerosis*, 43, 39, 1982. With permission.)

tendency to fibrosis, hyalinization, and hyaline swelling. Various degrees of fragmentation, distorsion, fraying, and lysis of thick collagen fibers and of cellular disintegration could be seen. Some of these disintegrated structures in the deeper region of the plaque may undergo fatty changes which are included in the necrotic center of the lesion.

This important encrustation of microthrombi within fibronecrotic and fibrohyaline plaques accounted for the important increase in the degree of luminal narrowing induced by the respective plaques. On a 5-year age basis, the mean reduction in the luminal diameter by atherosclerotic plaques in apparently healthy subjects who died in traffic accidents was 58% in individuals aged 41 to 45 years and 68% in individuals aged 46 to 50 years, with important

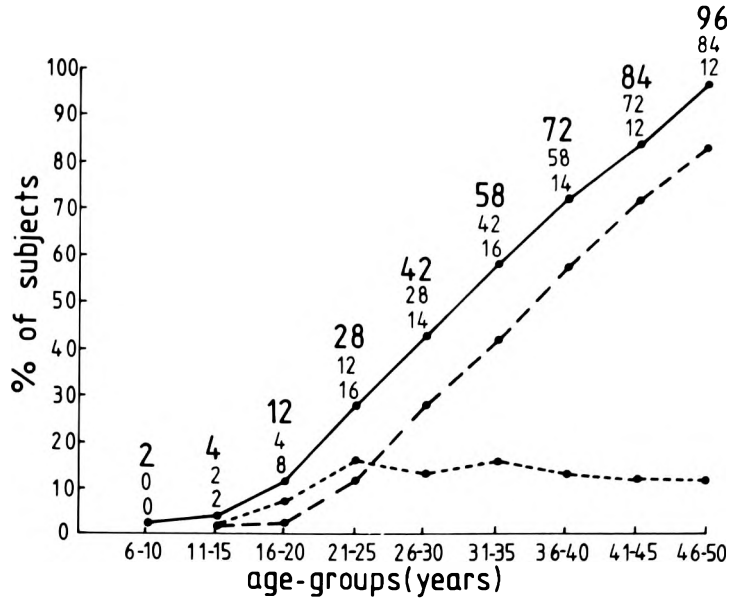

FIGURE 107. Percentage of subjects with coronary atherosclerotic plaques in successive 5-year age groups, from children to mature adults. (Solid line) Percentage of subjects with both preexisting and newly formed coronary atherosclerotic plaques; (broken line) percentage of subjects with suggested preexisting plaques; (dotted line) percentage of subjects with suggested newly formed plaques.

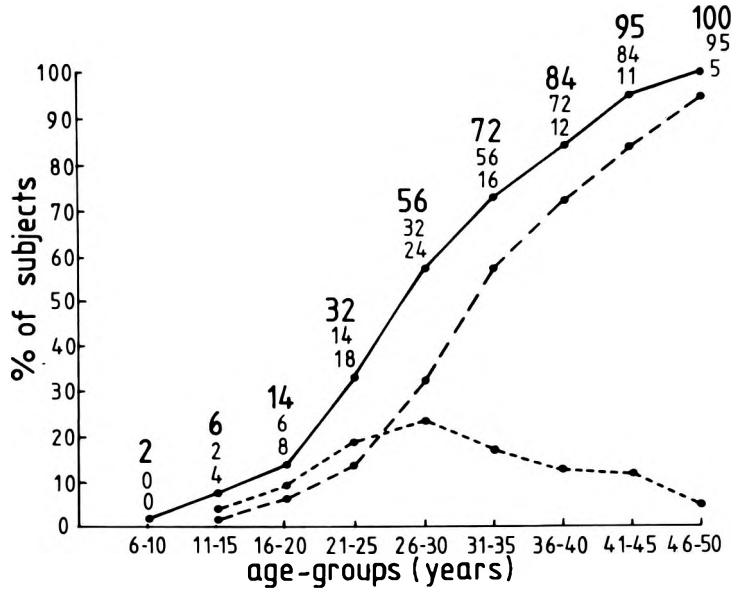

FIGURE 108. Percentage of subjects with coronary gelatinous lesions and intimal necrotic areas in successive 5-year age groups, from children to mature adults. (Solid line) Percentage of subjects with both preexisting and newly formed coronary gelatinous lesions and intimal necrotic areas; (broken line) percentage of subjects with suggested preexisting gelatinous lesions and intimal necrotic areas; (dotted line) percentage of subjects with suggested newly formed coronary gelatinous lesions and intimal necrotic areas.

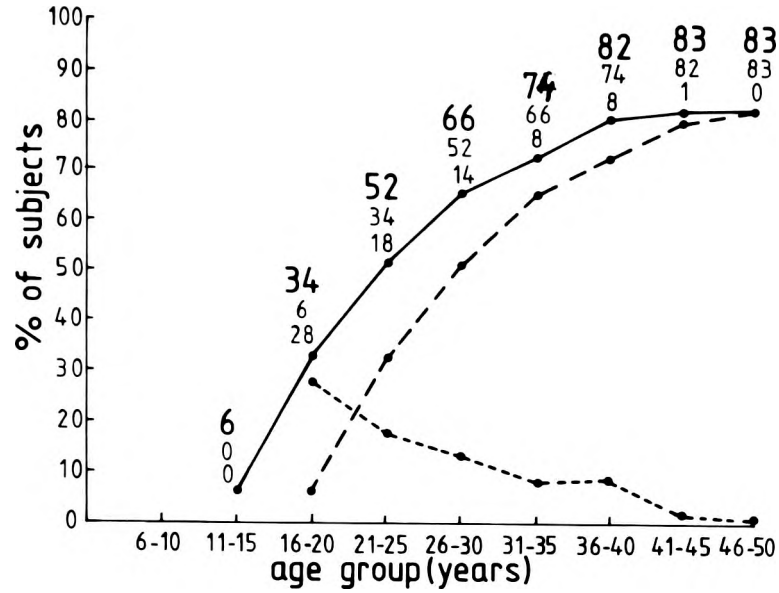

FIGURE 109. Percentage of subjects with coronary fatty streaks in successive 5-year age groups, from children to mature adults. (Solid line) Percentage of subjects with both preexisting and newly formed coronary fatty streaks; (broken line) percentage of subjects with suggested preexisting coronary fatty streaks; (dotted line) percentage of subjects with suggested newly formed coronary fatty streaks.

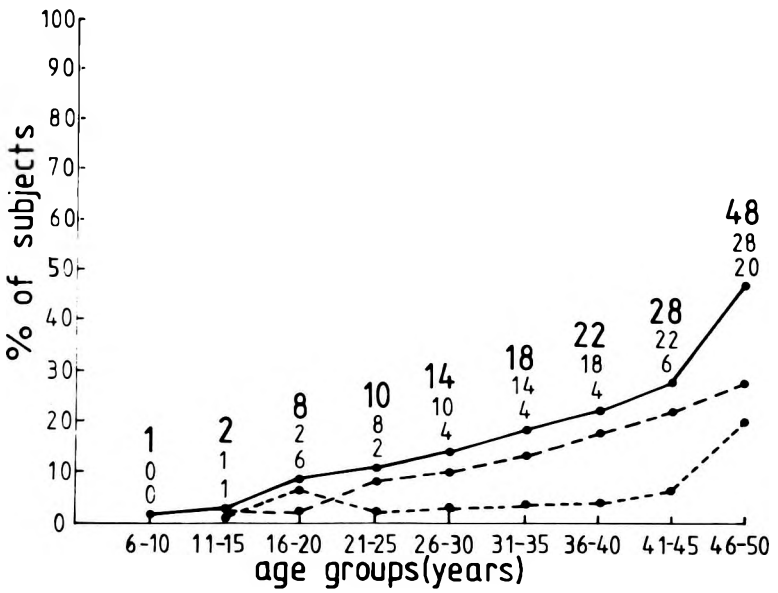

FIGURE 110. Percentage of subjects with coronary incorporated microthrombi and intramural thrombi in successive 5-year age groups, from children to mature adults. (Solid line) Percentage of subjects with both preexisting and newly formed coronary incorporated microthrombi and intramural thrombi; (broken line) percentage of subjects with suggested preexisting coronary incorporated microthrombi and intramural thrombi; (dotted line) percentage of subjects with suggested newly formed incorporated microthrombi and intramural thrombi.

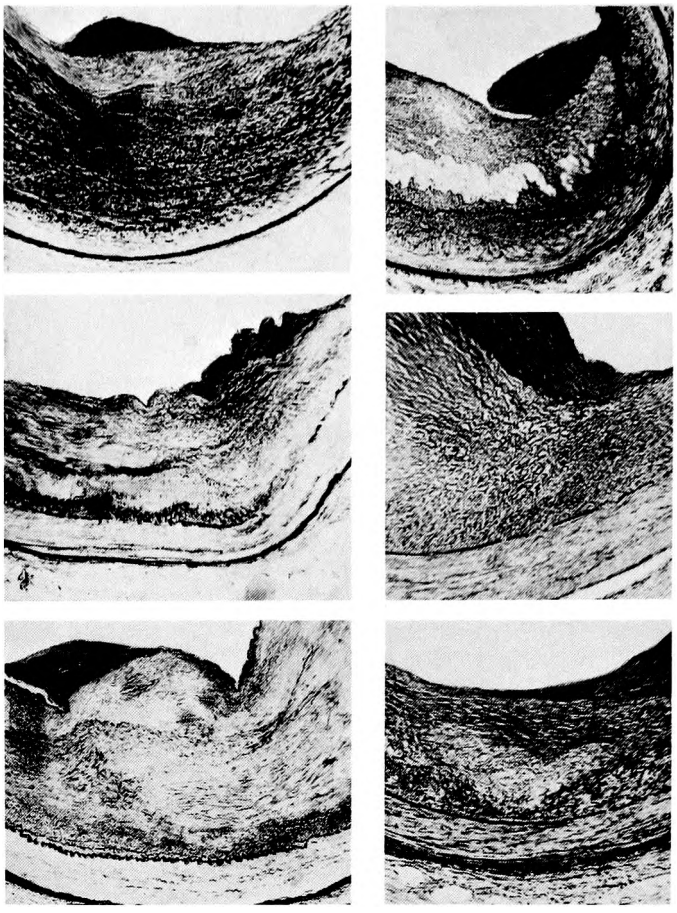

FIGURE 111. Microthrombi (in black) juxtapositioned on preexisting atherosclerotic plaques in subjects aged 41 to 50 years, especially in heavy smokers. (Lendrum's method for fibrin. Magnification × 120.)

sex differences: 79% for male and only 57% for female subjects aged 46 to 50 years. In the material investigated, *microthrombi juxtaposition and encrustation within preexisting fibronecrotic and fibrohyaline plaques appeared as the main cause of luminal narrowing and of the onset of lesions of possible clinical significance.* The continuous lipid deposition within necrotic centers of fibronecrotic plaques probably represents another obstructive mechanism, apparently of secondary importance in our unselected population sample.

We also noted that in the first diagonal, first septal, posterior descending, left, and right marginal arteries, as well as in the vessels supplying the conduction system, the number of subjects with atherosclerotic plaques aged 41 to 50 years was at least twofold greater than the number detected in the 31 to 40 years group. *The fifth decade appeared as the period of life in which the development of plaques of possible clinical significance is to be expected not only in the major coronary arteries, but also in their main branch vessels.*

Fibrin and platelet deposition on a stenotic coronary atherosclerotic plaque appeared in our material the most serious complication that can affect an advanced atherosclerotic lesion in subjects with an apparently normal lifestyle who died in traffic accidents.

It is generally assumed that occluding thrombi form because of rupture of the fibrous cap separating the luminal blood from the intraplaque necrotic center. The rupture of the fibrous cap allowing contact of the blood with the debris initiates a platelet-fibrin nidus, the mass of aggregate platelets augmenting and decreasing successively. This may be associated with the occurrence of peripheral emboli, and when fibrin accumulation becomes prevalent, the

thrombus acquires an occlusive character. These occluding thrombi were seen in the vicinity of small cracks or ruptures of the artery wall, which are often neglected by pathologists or are recorded post-mortem artifacts. In many works, *the association of wall rupture and occlusive thrombus is considered a cause-effect relationship.*[90] The wall rupture may be preceded by its invasion by lipid-rich macrophages which form a mantle of cells surrounding the central necrotic area.[164] It is difficult to establish, based on a succession of static pictures, whether these macrophages themselves had eroded and thus fractured the fibrous cap or whether they had been attracted by the erosion caused by some other factors.

Rupture of a plaque fibrous cap may also lead to hemorrhage into the plaque, discharge into the lumen of grumous material causing local vascular obstruction or peripheral embolization, and mural or occlusive thrombosis. A study of the connection between the presence of stenotic atherosclerotic plaques, intraplaque hemorrhage, plaque rupture, and thrombosis showed that if the stenosis was less than 75%, the rupture would cause hemorrhage without significant luminal thrombosis. On the other hand, with stenoses in excess of 75%, the formation of occlusive thrombi occurred with increasing frequency.[165]

To sum up, rupture of a plaque may cause both peripheral embolization and occlusive thrombus formation and, in addition, intraplaque hemorrhage.

It has been claimed that *plaque rupture may be considered as an incidental event in the evolution and growth of an atherosclerotic plaque* and not as an intrinsic part of its natural history. This rupture happens when fibrous cap softening develops so far as to fracture collagen fibers and bundles. Variations in blood pressure and vascular tone and particularly coronary vasospasm might be involved in plaque rupture. When a fragile cap is present, sometimes no more than the normal bending and twisting of the coronary artery which occurs during every heart contraction is needed to produce rupture.

An important question to be answered concerns the histolytic activity of some agents present in the necrotic core of the plaque. As shown in a previous section of this chapter, in subjects aged 41 to 50 years, extension of some necrotic centers by means of a border zone could be detected. The wavefront of necrosis usually involved the lateral side of the lesion in the direction of the blood flow, but in some plaques it also involves the luminal side and produces plaque rupture and ulceration. We found apparently stabilized necrotic centers with well-delineated frontiers and aggressive necrotic centers with peninsulas of necrosis invading the neighboring intimal connective tissue. This aggressive character might be related to plasma components accumulating in the necrotic center of the plaque, able to disintegrate very thick, hyalinized collagen bundles, thick elastic laminae, ground substance, and cells with or without the associated presence of an inflammatory infiltrate or of lipid-laden cells, as well as with or without the presence of newly formed capillaries from the advantitia or from the coronary artery lumen.

An intriguing aspect is the absence of intimal connective tissue response to the severe injury produced by the aggressive character of necrotic centers. This clearly demonstrates that the assumption that human atherosclerosis is nothing more than a reaction of the arterial wall to invading lipids might be a preconceived idea suggested by artificial experimental models.

In a study on the characteristics of the plaque under a coronary thrombus,[166] it was emphasized that all plaques with thrombosis had necrotic cores, that rupture and hemorrhage were found in 90% of thrombotic plaques, and that small cell infiltrates and atherophagocytosis were found in 72 to 94% of the thrombotic plaques. Also of note is the observation that thrombogenicity and its accompanying plaque cellularity are not incited by cholesterol, but by some traces or minor components of the plaque necrotic center. A new plaque component has been identified at the boundary between the necrotic core and the fibrous base of the plaque, occurring as a dense cluster of cells involved in atherophagocytosis. Small cholesterol crystals were contained within cells and large crystals were covered by

cells which seemed to flow together into a syncytium. These particular changes may differentiate thrombogenic from nonthrombogenic plaques.

In certain works, emphasis was placed on intraplaque hemorrhage.[167,168] In serially sectioned lesions with coronary thrombosis, intramural hemorrhage was identified in about 90% of plaques.[169] It was also suggested that thromboplastic substances resulting from hemorrhagic disruption of intimal connective tissue may initiate thrombosis. According to other views, intimal hemorrhage is indeed often present in atherosclerotic plaques complicated with thrombosis, but this is only an association without a causal relationship with the occlusive evolution of the lesion.

The basal part of the thrombus invariably located at the site of the wall rupture is the first submitted to connective tissue organization of an avascular type. This thrombus-replacing connective tissue springs from adventitial capillaries and enters the thrombus via the locus of the previous fibrous cap rupture. The blood vessels of this thrombus-replacing tissue seem to be especially prone to produce intraplaque hemorrhage. Certain investigators assumed that it is difficult to accept the idea of an intimal hemorrhage from a low pressure capillary source capable of becoming occlusive despite the high pressure existing in the coronary arterial lumen.[170]

An intriguing question is the frequency with which a nonobstructive thrombus superimposed on a preexisting stenotic plaque is subject to repeated build-up and dissolution cycles. Myocardial clinical manifestations may occur without the presence of an obstructive thrombus, but as a result of embolism from the surface of the respective thrombus. There is reason to presume that coronary mural thrombosis developing in relation to an advanced atherosclerotic plaque does not differ in any significant way from the same process in the carotid or vertebral arteries.[171] Platelet aggregates may give rise to transient ischemic attacks in both brain and myocardium and they may produce transient monocular blindness and fatal arrhythmias. It is possible that one of the causes of sudden cardiac death without apparent obstructive lesions in the major coronary arteries is the showering of the intramyocardial vessels by platelet aggregate emboli released from a developing mural thrombus. This view is supported by the observations carried out on experimental models which showed that an intraarterial thrombus repeatedly builds up to a critical size, breaks off and is then carried downstream. This tendency to form platelet aggregates was seen to increase significantly following epinephrine injection and to decrease after dipyridamol and aspirin treatment.[172] This could be related to the massive release of catecholamines with the possible clumping of platelets in the myocardial circulation during repeated and severe psychosocial stresses and may help to explain certain sudden cardiac deaths which occur after such stresses. In these cases without thrombotic occlusion or even severe stenotic atherosclerotic plaques in the major coronary arteries, an apparently insignificant mural thrombus could be a source of platelet emboli, producing arrhythmias and sudden cardiac death. In all cases, when thrombus projects into but does not totally occlude the lumen of an artery, the potential exists for emboli to pass distally into the segment of myocardium supplied by that artery.[173] Such emboli may be responsible for myocardial clinical manifestations occurring in the crescendo form of unstable angina pectoris (particularly when it culminates in acute myocardial infarction) and various forms of lethal arrhythmias. Segments of myocardium that were not supplied by an artery containing mural thrombi which releases platelet aggregate emboli seem to be spared from reversible transient obstruction of such small intramyocardial vessels.

Pathologic studies can demonstrate the presence of these platelet emboli but cannot give an answer as to whether they are responsible for ill effects. Establishing cause-effect relationships in post-mortem studies remains a difficult task for every pathologist.

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Chapter 5

THE END STAGES OF THE NATURAL HISTORY OF CORONARY ATHEROSCLEROSIS

I. INTRODUCTORY REMARKS

A. Risk Factors' Involvement

1. Risk Factors and Coronary Atherosclerosis

In spite of many uninterrupted efforts, we could not relate in our material the onset of fibromuscular plaques, gelatinous lesions, intimal necrotic areas, fatty streaks, incorporated microthrombi, and intramural thrombi, and also the occurrence of very thick intimas to the preexistence of a particular risk factor or constellation of factors. Many early atherosclerotic lesions develop in subjects without the so-called "cardiovascular risk".

On the other hand, the use of the method of successive microscopic observations of similar topographic sites placed in sequence according to age, sex, and anatomical branching pattern allowed us to obtain a dynamic view from many static aspects as concerns the progression of early lesions toward advanced lesions. Based on the results furnished by this method we could demonstrate that both endogenous and exogenous risk factors accelerate the rate of progression of preexisting lesions. Moreover, they might give rise to particular cycles of evolution toward lesions of possible clinical significance.[1]

An effort has been also made in our institute to delineate an index of prognosis, related to the progression of coronary atherosclerotic plaques toward these advanced lesions involved in myocardial clinical manifestations. In this respect, a synthetic index for prognosis was suggested: methods of multivariate mathematical statistics were applied for combining a set of simultaneous characteristics (risk factors) related to the onset of myocardial clinical manifestations induced by coronary atherosclerosis into a single representative value as index of the individual state.[2] Successive values of this index give the time-dependent changes of the individual state of health.

We hasten to add that the underlying mechanisms by means of which risk factors accelerate the rate of evolution of coronary atherosclerotic lesions are still the subject of debate. Until this matter is resolved, the reader is cautioned to assess critically the current literature before drawing a conclusion. We are not among the investigators who consider that risk factors are the *sine qua non* of human coronary atherosclerosis and we do not rely only on macroscopic sudanophilia to detect coronary atherosclerotic lesions. On the other hand, we are firmly convinced that some risk factors are operative in certain subjects and not in others, in some arterial beds and not in other arterial beds, in some arterial segments of a given vascular tree and not in others. We can do nothing about risk factors such as age, male sex, anatomical branching pattern, and family history; on the other hand, we can alter the exogenous risk factors, such as smoking. It might be of interest to note that in our material the number of coronary atherosclerotic plaques with incorporated microthrombi increased in mature adults up to 3.6 times from one 5-year age group to the next in heavy cigarette smokers and only up to 1.4 times in nonsmokers. Our investigations, based on the method of successive observations of similar topographic sites revealed that *whereas the general rate of progression of early atherosclerotic lesions toward advanced ones appear to be largely age-dependent, the acceleration of this rate and the occurrence of particular cycles of evolution seem to be largely risk-factor dependent.*

In the absence of the major risk factors, a limited number of fibromuscular plaques of children and juveniles are converted to mucoid plaques during adolescence. In the presence of tobacco smoking (10 to 12 cigarettes daily), this number increased significantly. Likewise,

in the absence of the major risk factors the progression of fibromuscular plaques toward the fibronecrotic is very complex, including as intermediate stages mucoid, foam cell-rich, and necrotic plaques. In the presence of risk factors this progression is not only accelerated, but also short-circuited, a direct passage from fibromuscular to fibronecrotic plaques being deduced from many static aspects placed against age. The use of the above-mentioned method also revealed that while some lesions are still progressing, others seem to have stopped growing, and even in the same coronary arterial tree, there may be different rates of lesion progression in the presence of a similar major risk factor or constellation of factors. In fact, from the 10 to 12 atherosclerotic plaques usually detected in the coronary arterial bed of a middle-aged adult man, only 1 or 2 might be regarded as being of possible clinical significance, but this proportion augmented in the presence of operative risk factors. In our institute, the probability of developing myocardial clinical manifestations induced by the progression of early to advanced lesions within a definite time interval under the influence of operative risk factors was investigated by means of multiple logistic function.[3] The coefficients were calculated from the data furnished by risk factor measurements, carried out in a 10-year longitudinal study on 5000 male subjects from 40 to 60 years old.[4,5] Individual risk of myocardial clinical manifestations varied from 0.08 to 0.63% for the entire population, the greater number of cases with myocardial clinical manifestations occurring in subjects considered to be at high risk. First in importance among the risk factor variables was systolic blood pressure, followed by the number of cigarette smoked, age, and serum total cholesterol levels.[5] Also of note is the observation that an optimal rhythm of interventions seems to exist, according to the nature and intensity of risk factors. This is reflected by the fact that periods of response to active interventions were followed by periods of inertia.[6]

If all plaques would progress and never stopped growing, the lumen of all major coronary arteries and main branch vessels in all mature adults would be completely occluded in each individual submitted to the action of the major risk factors. In fact, a very limited number of early lesions progress to advanced ones and this demonstrates that this progression would depend not only on the mere presence of risk factors, but on a complex of local conditions which determine that the respective risk factor is operative only at the level of one or two out of more than ten plaques. A number of lines of evidence support the view that in the presence of many risk factors, only certain of these risk factors in particular periods of life and pathophysiologic conditions are operational.

Moreover, the same risk factor seems to act differently in various segments of the coronary arterial tree: it may accelerate the progression of fibromuscular plaques in some sites, of gelatinous lesions in other sites, and it may favor the onset of intramural thrombi or the development of a very thick intima.

As already noted in Chapter 4, coronary atherosclerosis and coronary heart disease are strongly related but not similar pathologic entities: each has its own etiology and pathogenetic mechanisms. Both of these pathologic entities are multifactorial diseases and there is a tendency in many places to ignore this complexity, to single out one major risk factor (usually hypercholesterolemia) and to attempt to explain everything else as a result of the quantitative variations of that one risk factor. This is a dangerous trap, since in the absence of risk factors many individuals may present a very short natural history of coronary atherosclerosis (Hurler's syndrome, Kawasaki disease, etc.) with a rapid onset of myocardial clinical manifestations. On the other hand, in the presence of all three major risk factors (hypertension, hypercholesterolemia, and tobacco smoking), many individuals have a very long and asymptomatic natural history of coronary atherosclerosis and the available literature shows that less than 50% of myocardial clinical manifestations considered to be induced by coronary atherosclerosis can be explained if risk factors are involved in the main pathogenetic mechanisms.

The age- and risk factor-related progression of coronary atherosclerotic lesions emphasized

in our results[1] were also mentioned by other investigators. Thus, in a study carried out in Japan, the severity of coronary atherosclerosis as revealed by the degree of luminal narrowing showed both an age- and risk factor-related progression.[7] The severity of coronary atherosclerosis appeared first related to age, but also correlated positively with antecedent elevated blood pressure and with higher serum total cholesterol levels. The overall contribution of seven selected risk factors to coronary atherosclerosis was 33.5% of the total variation by the analysis of the multiple regression model.[7]

Data furnished by the International Atherosclerosis Project[8] and other autopsy surveys can also be used to illustrate the influence of risk factors on the progression of coronary atherosclerotic lesions. Advanced lesions were found 4.4 times more frequently in men who smoked two or more packs of cigarettes per day than in men who never smoked regularly.[9] Likewise, advanced hyaline thickening of the arterioles in the myocardium was presented in certain works as a characteristic change associated with smoking.[10] It has already been mentioned that in our material, smoking was associated with a frequent occurrence of mucoid plaques in adolescents and young adults.[11] This occurrence was related to the massive insudation produced by smoking in preexisting fibromuscular plaques. In the Oslo study, smoking habits were not statistically associated with the occurrence of advanced stenotic plaques in the coronary arteries, whether this association appeared with serum cholesterol, systolic, and diastolic blood pressure.[12] This study also showed that selected risk factors did not seem to have any independent influence on the development of coronary stenoses over and above that of the extent of raised atherosclerotic lesions. These observations support the hypothesis that risk factors work through the development of raised lesions to produce stenotic lesions; once developed, these stenotic lesions increase the risk of myocardial clinical manifestations.

In a Hong-Kong pathologic study, aggregation of all subjects into smokers and nonsmokers did not reveal much difference either in the incidence of coronary atherosclerosis or the degree of narrowing.[13] This and many other studies suggest that cigarette smoking has a more complex action on the coronary arterial wall than that of the progression of raised lesions. Thus, for instance, platelets were found to be more adhesive to the vessel wall in smokers,[14] and nicotine has been found to decrease prostaglandin (PGI_2) synthesis in endothelium.[15] Likewise, chronic smokers have reduced PGI_2 levels in the systemic circulation and coronary sinus as compared to nonsmokers.[16] This complex action of cigarette smoking seems to be reflected also by the variety of coronary artery wall changes.[17]

In the Framingham study, the relation of antemortem characteristics to cardiovascular findings at necropsy appeared peculiar.[18] The failure to find a strong, consistent correlation between serum cholesterol levels and severity of atherosclerotic involvement was mainly demonstrated in women. On the other hand, both systolic and diastolic blood pressures recorded 9 years before death were positively correlated with all degrees of coronary atherosclerosis in women. In males, few pathologic correlations were found with blood pressure, whereas serum cholesterol 1.5 and 9.0 years antemortem all correlated positively with the degree of luminal narrowing.

In the prospective Oslo study, the post-mortem data were also compared with risk factors recorded before death and the results revealed that serum cholesterol levels, blood pressure levels, and number of cigarettes smoked could explain only 20% of the variation in raised lesions — about 80% remained unexplained.[19]

An investigation of coronary risk factors and autopsy findings in Japanese-American men[20] revealed a statistically independent association between coronary atherosclerosis, on the one hand and relative weight, cigarettes per day, and serum cholesterol levels, on the other hand. These risk factors seem to favor the progression of certain plaques toward obstructive lesions. A similar conclusion resulted from an investigation on post-mortem serum cholesterol and the extent of intimal surface involved by atherosclerotic lesions visible on gross inspection in men aged 25 to 44 years resident in Orleans Parish, Louisiana.[21]

There is some evidence suggesting that a certain risk factor can favor a more operative influence of other risk factor on the progression of atherosclerotic lesions. Thus, for instance, high serum cholesterol levels could increase the severity of lesions produced by arterial hypertension.[7] Many other data on this atherogenic action of arterial hypertension have already been discussed in Chapter 3.

The influence of risk factors on the onset and progression of coronary atherosclerotic plaques was largely investigated angiographically. The results are as diverse as fingerprints and some of these results showed that risk factors at the initial catheterization have not been found helpful in determining which cases will progress to lesions of possible clinical significance.[22-25] Angiographic progression of lesions was in certain investigations more clearly associated with decreasing left ventricular function than with risk factors at first and second catheterization.

An attempt to sort out from many observations the evidence that seems to be more closely related to the natural history of coronary atherosclerosis leads to the view that *risk factors cannot be considered as* sine qua non *for the onset and progression of atherosclerotic lesions. Atherosclerotic lesions develop either in the absence or in the presence of risk factors, but their progression toward lesions of possible clinical significance is accelerated by hypertension, hypercholesterolemia, and smoking.* Since early atherosclerotic lesions continue to appear in the coronary arterial bed from childhood to late adulthood, a similar risk factor, acting with a similar intensity, may influence an early fibromuscular plaque which develops in the atrioventricular node artery of a mature adult man, an advanced fibronecrotic plaque present in the proximal segment of the left anterior descending artery, an intramural thrombus which forms at the branching site of the first septal vessel, and many other lesions existing in various vascular segments of the same coronary arterial tree. It is not possible to deduce by our present methods of investigation on which lesion the respective risk factor is more operative. The simplistic view that all early atherosclerotic lesions develop during childhood and are then submitted to the action of risk factors is unrealistic and must be revised.

In the unselected Bucharest population sample investigated during the last 2 decades two endogenous risk factors appeared of particular importance in the natural history of coronary atherosclerosis: the anatomical branching pattern of the coronary arteries and the male sex.[26,27] The onset of the first fibromuscular plaques in the coronary arteries of children was clearly associated with the presence of some minor atherogenic deviations from the common type of distribution of the coronary arteries. Many data on this association can be found in Chapters 2 and 3. As concerns the influence of sex, the first atherosclerotic plaques appeared in male children about 1 decade earlier than in female adolescents. Starting from adolescence, the sex ratio for positive cases with atherosclerotic plaques became statistically significant and the men:women ratio of cases with necrotic and fibronecrotic plaques was 2.6 in the subjects 26 to 30 years old, 2.4 in those 31 to 35 years old, and 2.0 in the subjects 36 to 40 years old. This means that at a similar age, men had two- or threefold more numerous advanced lesions than women. In each 5-year age group, the intima of the left anterior descending and left circumflex arteries was thicker in males than in females, but no statistically significant sex differences appeared in the right coronary artery.[27] In essence, in our material, differences in morbidity rates from coronary heart disease in adult males vs. adult females could be partly explained by sex differences in the natural history of coronary atherosclerosis.

2. Risk Factors and Coronary Heart Disease

Systematic epidemiological investigations to detect risk factors for coronary heart disease and to prevent myocardial clinical manifestations induced by coronary atherosclerosis started soon after the end of World War II. In the course of these more than 4 decades, much has been learned and also much remains to be discovered.

In spite of many uncertainties which persist, an adequate control of the risk factors is the only recommendation that should be utilized by practitioners. When properly instituted, positive results can be obtained in some persons by lowering elevated blood pressure and serum cholesterol levels, or after cessation of cigarette smoking, associated with dietary management of weight control, a rational program of physical activity, and also diminution of the intensity of psychosocial stresses. It is our duty to remember the initial call for an effort to prevent coronary heart disease, co-authored in 1959 by White et al., who emphasized the role of environment in the incidence of end points of the natural history of coronary atherosclerosis.

During these attempts for primary prevention, cities such as Framingham, Oslo, Tecumseh, Bogalusa, etc. have often been mentioned in the available literature, as well as many studies carried out under World Health Organization (WHO) authority. In 1971, WHO convened an international meeting where different strategies were proposed involving randomized trials in high risk groups with uni-, bi-, and particularly multifactorial risk interventions. The results of all these complex studies provided us with evidence of an associative and in some instances a predictive relationship to myocardial ischemia of elevated blood pressure, excessive cigarette smoking, raised total serum cholesterol and LDL-cholesterol (low density lipoprotein), low HDL-cholesterol, (HDL: high density lipoprotein), hypertriglyceridemia, obesity, diabetes mellitus, male sex, parental history of coronary heart disease, electrocardiographic abnormalities, inadequate exercise, the prevalence of the type A behavior pattern, psychosocial stresses of various forms and intensities, and many other risk factors, some of which are considered established, others, controversial.

The results of these complex studies revealed that the passage from occult to symptomatic myocardial ischemia with its various forms of clinical manifestations is more frequently detected in populations with habitual diets rich in calories, total fat, saturated fat, cholesterol, and refined carbohydrates. An additional basic conclusion is that the incidence rate of myocardial clinical manifestations induced by coronary atherosclerosis varies directly with economic well being. This positive correlation between mortality rates from coronary heart disease, on the one hand, per capita income, mode of life, and economic development, on the other hand, is an important result of epidemiologic studies. These results represent the starting point of many programs aimed at preventing myocardial clinical manifestations, the necessity of such programs being often defended on the basis of our urgent need to do something even when our knowledge is incomplete.

While not denying the importance of epidemiologic studies, we should adopt a cautious attitude when some investigators overemphasize their importance and try to explain a large area of human pathology called coronary heart disease solely by means of quantitative variations of risk factors. The epidemiological approach measures the myocardial clinical manifestations induced by coronary atherosclerosis in terms such as ''prevalence of clinical manifestations'', ''incidence of various aspects of these clinical manifestations'', ''loss of life'', etc. Epidemiological studies have totally neglected the existence of silent myocardial ischemia, although this could represent in certain populations an important stage in the natural history of coronary atherosclerosis. If, for instance, in a selected population sample 10% of cases have silent myocardial ischemia, 100 of 1000 subjects are erroneously considered normals since they do not exhibit myocardial clinical manifestations. A subject asymptomatic at entry to the study is not equivalent with a normal subject. Many of these normal cases had severe coronary atherosclerotic involvement with stenotic or even obstructive plaques bypassed by adequate collateral vessels. The asymptomatic population submitted to the study of the effect risk factor intervention is not a homogeneous one, but a heterogeneous population concerning the topography and severity of atherosclerotic plaques. The effect of risk factor change on this heterogeneous population is difficult to establish and the concept that this population is free of coronary disease is unrealistic. The existence of silent

myocardial ischemia and the lack of specificity of some of the clinical end points used in epidemiologic studies, such as angina pectoris or sudden cardiac death, may mask some important associations between risk factors and myocardial ischemia. Whereas the rate of progression of coronary atherosclerotic lesions in subjects with silent myocardial ischemia under the influence of risk factors is significantly underestimated, in symptomatic myocardial ischemia overestimation is the rule. Progression from silent to symptomatic myocardial ischemia occurs in relation to time and as the interval between examinations increases, there is a tendency to record more important actions of risk factors.

Epidemiological studies, associated with angiographic and pathologic findings, have conclusively shown that in populations where there is a high risk of myocardial infarction and sudden cardiac death, there is also a severe atherosclerotic involvement of the coronary arteries. They have also shown that coronary atherosclerosis rarely gives rise to myocardial clinical manifestations until the fourth decade of life is reached; by the fifth decade 1 to 2% and by the sixth decade 3 to 4% of the male population of industrialized countries have clinical manifestations of coronary heart disease. Approximately 50% of all deaths appear in many epidemiological investigations as being due to myocardial clinical manifestations considered to be induced by coronary atherosclerosis.

The capacity attributed to risk factors to aggravate the end stages of the natural history of coronary atherosclerosis is presented in many works as being causative. It is not intended to give a detailed account of the literature upon this subject, but from many publications it is evident that cessation of smoking was followed in certain selected samples by a diminution of up to 50% of the incidence of myocardial clinical manifestations.[28] One of the most important effects of cigarette smoke is to impair endothelial PGI_2 production;[29] an inefficient synthesis of endothelial PGI_2 in heavy smokers may lead to a progressive loss of endothelial thromboresistance, increased concentrations of plasma epinephrine and norepinephrine, factor XII, and many other changes.

Can we accept that certain of the changes produced by cigarette smoking are causative for the onset of the early atherosclerotic lesions, others for the progression from early to advanced lesions, plaque hemorrhage, rupture and thrombosis of advanced lesions, and embolism in the coronary circulation? Cigarette smoking is also a risk factor able to act on myocardial cells, cells of the conduction system of the heart, and cells of the nerves and ganglia of the heart. On the other hand, we are unable to produce atherosclerotic lesions in laboratory animals submitted to the action of cigarette smoke.

In addition to "the big three" (blood pressure level, serum cholesterol level, and smoking) which still hold center stage, type A behavior is also regarded among the primary causal agents in the pathogenesis of coronary heart disease.[30] Like smoking, its action on the cardiovascular system seems to be very complex and difficult to delineate. Type A behavior consists of many components, some overt (hyperaggressiveness, free-floating hostility, and sense of time urgency), others hidden (inadequate self-esteem and/or insecurity and a drive toward self-destruction). Certain results suggest that it was possible to reduce the intensity of type A behavior in the majority of postinfarction subjects and that such behavior modification was found to lead to marked protection against a recurrent coronary event.[31]

Association sometimes means causation and for some aspects of lifestyle causative relationships have been demonstrated between risk factors and coronary heart disease.[32] Questions have been raised as to the validity of such views and many investigators believe that the data are not precise enough to be convincing. It would appear from certain of these data that risk factors are only fellow travelers without a significant implication in the pathogenetic mechanisms of coronary heart disease: "the contribution of the risk factor concept to patient care in coronary heart disease has been and still is trivial".[33] Severity of angina pectoris and the history of past myocardial infarction are by far more important in predicting future risk than the sedentary lifestyle or the automobile-TV society. It probably is also worth

noting that "it is perhaps naive to expect that the measurement of the plasma concentration of either plasma lipids or apoproteins would be strongly related to the severity of atherosclerotic lesions".[34] The evidence derived from other studies shows that LDL-cholesterol and apoprotein B levels are strongly related to the severity and extent of coronary atherosclerosis, but not to the number and severity of angiographically distinct stenoses.[35]

Although perhaps too simplistic and overemphasized by some, epidemiological investigations have stimulated much basic research and clinical studies. We have recently reviewed a wide variety of documents from these epidemiological investigations on various risk factors believed operative in the onset of the end stages of the natural history of coronary atherosclerosis. It is appropriate to question whether the scientific foundation of some of these studies is adequate and if it is realistic to maintain that a quantitative change in certain risk factors is the answer to atherogenesis, progression of atherosclerotic lesions, and passage from asymptomatic to symptomatic stages of the natural history of coronary atherosclerosis. Epidemiologists overlook the fact that the pathogenetic mechanisms of risk factors are not clearly understood and we do not know why some actions are additive, with the risk increasing disproportionately when more than one risk factor is present. Epidemiologists also frequently overlook the fact that many victims of myocardial infarction or sudden cardiac death do not exhibit different levels of risk factors or differences of habitual diet, compared to subjects without myocardial clinical manifestations in longitudinal studies. Likewise, they are unable to explain why one or another risk factor shows high levels in various populations in which both myocardial infarction and sudden cardiac death remain relatively rare and why the same combination of risk factors appears associated with different rates of myocardial clinical manifestations in different populations. A sizeable proportion of people with multiple risk factors do not develop these myocardial clinical manifestations and, as already mentioned, the available literature demonstrates that less than half of the heart attacks in a population can be associated with risk factors for coronary heart disease.

All the evidence presently available indicates that the predictive power of a risk factor for coronary heart disease is very difficult to establish in the presence of many atherogenic and thrombogenic factors. Indirect evidence is more demonstrative to obtain, trying to predict if a reduction in a given risk factor will reduce the risk of coronary heart disease in the entire population, population groups, or in specimens chosen in a variety of ways. Some intervention studies have demonstrated, for instance, that serum cholesterol reduction in subjects with abnormal levels is a precondition for lowering the incidence of coronary heart disease, and the Lipid Research Clinic Coronary Trial[36,37] is an eloquent example in this field. There is no excuse for not starting to apply now in the way of prevention what has been established with reasonable and adequate accuracy. The results of the study mentioned have offered definitive evidence of the benefit of lowering cholesterol, in spite of the fact that these conclusions are limited only to adult individuals with elevated serum cholesterol levels. From the evidence provided, it appears that serum cholesterol concentration is an important risk factor for coronary heart disease at levels of 260 mg/dℓ or more. This evidence advocates for a policy of selective intervention, but its intracation with mass intervention remains debatable.[38-42] At present, it is safe to say that we lack solid scientific evidence that except for selected cases at the extreme ranges, dietary modifications, reduction of cigarette smoking, and treatment of arterial hypertension will prevent heart attacks in all countries, all population groups, and in all individual cases.

The scientific evidence supporting intervention for those at high risk for coronary heart disease due to the action of major risk factors is much stronger than for those at moderate or low risks. Some eminent authorities in the field of cardiovascular epidemiology give an affirmative answer to the question, "Can changes in lifestyle prevent atherosclerotic disease?"[43] It is reasonable to believe that to encourage a prudent diet, to abrogate smoking, to have a normal blood pressure level, ideal weight, and a lifetime commitment to exercise

may be recommended as a low-risk strategy. The prevalent view must be that in spite of the fact that we lack a definitive study on whether eating fewer eggs will prevent coronary heart disease,[44] we must accept lifestyle changes as good preventive medicine without solid scientific proof of efficiency. We can hope that quitting smoking, lowering the blood pressure, modifying the diet to reduce serum cholesterol level, exercising more, and reducing psychosocial stresses is a better health policy than "having a hot dog and beer and then smoking a cigarette while sitting in front of the TV on a Saturday afternoon".[45]

3. Risk Factors as Related to Secular Trends

Understanding secular trends in the natural history of coronary atherosclerosis and particularly in the occurrence of lesions of possible clinical significance is of special importance in establishing causal links between coronary atherosclerosis and coronary heart disease.

Epidemiological studies in different countries of the world and in different population groups within the same country have clearly revealed that myocardial clinical manifestations due to coronary atherosclerosis are more prevalent in the U.S., Canada, U.K., and Finland than in Sweden, the Netherlands, or Switzerland and far more prevalent in the former countries than in Japan, Ceylon, India, or China.

The age standardized mortality from coronary heart disease in men aged 35 to 64 years varied from 466.8/100,000 population in Finland to 73.3 in Japan. Post-mortem studies showed that coronary atherosclerotic involvement in the white population was high in New Orleans and Oslo, medium in Sao Paulo, and medium-low in Puerto Rico, Costa Rica, and Santiago. Atherosclerosis in the black population was medium-high in New Orleans, medium-low in Jamaica and Puerto Rico, and low in São Paulo.[46]

Important differences up to tenfold in the frequency of myocardial clinical manifestations were detected among men of the same age in different cultures. Not only did the high incidence of coronary heart disease appear as a remarkable natural experiment in Finland, but also the low incidence in the Japanese population and in parts of the Mediterranean basin.

It becomes apparent that coronary heart disease rates of specific ethnic groups differ from those of other groups to an extent that cannot be explained by the known risk factors. There were also unexplained differences among states of the same country: age-adjusted death rates (males 1968 to 1972) for coronary heart disease for North Carolina, Georgia, and South Carolina seemed to be 1 $^1/_3$ times greater than rates in New Mexico, Utah, and Colorado.[47] Framingham residents showed about twice the rate of myocardial infarction and sudden cardiac death as Europeans having statistically an equivalent distribution of standard risk factors for coronary heart disease. Likewise, the mortality from coronary heart disease for males aged approximately 40 was three times greater in Edinburgh than in Stockholm, whereas the serum cholesterol level of the two populations was similar. On the other hand, in Tashkent (U.S.S.R.), coronary heart disease incidence was found to be the lowest, while the spread of risk factors was practically the same or even higher than in other cities.[48] Another intriguing fact is the coronary heart disease mortality rate of middle-aged males which was twice as high in Finland as in Norway and Sweden, whereas the severity of coronary atherosclerotic involvement seemed to be similar.[49,50] Neither have differences been found in the study of the International Atherosclerosis Project between the severity of coronary atherosclerosis in the U.S. Whites and Norway, but the mortality rate of coronary heart disease in the U.S. was almost double that of Norway.[8]

Beginning in the 1940s in some countries, and the 1950s, 1960s, and even 1970s in other countries, coronary heart disease mortality rose dramatically year by year and became the leading cause of death. This mortality was not only very high, but also premature, since many victims were among middle-aged and even young adults. By the end of the seventh decade, a surprising epidemiologic phenomenon appeared after decades of increase: mortality

rates from coronary heart disease began to decrease, the U.S., Australia, New Zealand, Finland, Norway, Belgium, and Israel recording a declining coronary heart disease mortality. In many other countries, the prevailing trend for working-age men during the 1970s and 1980s has been a rise in male coronary heart disease mortality. In Sweden, for instance, coronary heart disease mortality increased by 30% among 50- to 54- year old men and by 20% among 55- to 59- year-old men between 1968 and 1980.[51] During the period in which mortality increased by 20 to 30% among men, there was no change in female coronary heart disease mortality.

Many investigators ask why secular trends in the frequency of myocardial clinical manifestations induced by coronary atherosclerosis are changing before our very eyes. It is important to emphasize that several countries have registered an important decline in coronary heart disease mortality rates, other countries no changes and finally other countries rising rates. The fall in coronary heart disease rates is not universal, but when it occurs it seems to be real, since in the U.S., for instance, mortality declined steadily in each successive year in both sexes of all age groups and all race groups.[52] Since the late 1960s death rates in the U.S. from coronary heart disease have fallen steadily and markedly (by over 30%) and this decline has continued into the early 1980s.[32] In Australia, for most age and sex groups, a peak in coronary heart disease mortality rates occurred from 1965 to 1967 and these mortality rates have been at their lowest level for the past 3 decades.[53]

The fall in coronary heart disease rates is real since each of the major subcategories of cardiovascular diseases also declined each year during this period, not only myocardial infarction. Of note is the decline of cerebrovascular disease, arterial hypertension, rheumatic heart disease; noncardiovascular causes of death also declined in the U.S. during the last 2 or 3 decades, the main exception being malignant neoplasms.

The following possible causes have been taken into consideration to explain the observed changes in mortality and morbidity:[54]

1. Reduction of risk factors for coronary heart disease
2. More rapid delivery of acute coronary care because of improved emergency medical services
3. Improved medical care for acute myocardial infarction during hospitalization
4. Improved medical care for chronic coronary heart disease both during and after hospitalization

This could explain the improvement of all indicators related to death rates outside the hospital, death rate in the hospital, hospitalization rates for acute myocardial infarction, hospital case fatality rates for acute myocardial infarction, etc.

In certain countries and population samples it is clear that alteration of risk factors, the emergency medical services, and coronary bypass surgery have contributed to the decline; on the other hand, in other countries this contribution is slight, of insignificant value. This leads some investigators to believe that the decline in coronary heart disease mortality may be associated with the absence of a major influenza epidemic during the last decades, as well as to the large use of β-blockers, calcium antagonists, and other drugs. An important argument against the effect of the use of antihypertensive drug therapy and of improved medical services is the observation that in many countries the most important advances are introduced after the beginning of the decline.

Certain scientists have speculated the decline in coronary heart disease rates as a proof that the right measures have been applied to control the epidemic of coronary heart disease. There is reason to believe that a decline in the incidence of myocardial clinical manifestations induced by coronary atherosclerosis points toward the efficiency of primary prevention, while a decline in mortality with no change in incidence would indicate the predominant

influence of factors related to medical care. The point is that in all industrialized countries a more or less similar strategy of general recommendations for the whole population and especially for those at higher risk was at work. In all these industrialized countries, physicians have contributed greatly to the treatment of hypertension, to the smoking cessation, better eating, exercise habits, reduction of psychosocial stress, etc. The available right measures have been taken to control the epidemic of coronary heart disease, but for reasons which are not clear, only in some countries or population samples has this control been associated with a decline of coronary heart disease mortality rates.

It is noteworthy that in Israel, for instance, the decline in coronary heart disease mortality was not associated with putatively anti-atherogenic trends in eating habits. As mentioned in a study on this subject, changes in way of life and risk factors which were presented as the underlying cause behind the widely publicized and reviewed coronary heart disease mortality decline in the U.S. have hardly taken place in Israel, and constitute a totally unsuitable explanation for the declined death rates in Israel.[55] The swift decline in Israeli Jews occurred despite gradually increasing fat intake and reduced intake of nonrefined carbohydrates, and no apparent changes in cholesterol levels and cigarette smoking habits. In Switzerland, the coronary heart disease rate declined by 13% for men and 40% for women between 1951 and 1976, while the consumption of animal fats per capita increased by 20%; smoking also increased among women and did not decline appreciably among men. Fat consumption and serum cholesterol levels have increased in Japan at the same time as coronary heart disease mortality has been reported to decline.[28]

Many other examples can be given on the problem of secular trends of coronary heart disease, and all tend to demonstrate that we have evidence that neither an increase in risk factor levels and corresponding lifestyle are the main cause of an augmented coronary heart disease mortality, nor that declines in coronary heart disease mortality run parallel with improvements in the risk factor constellations and lifestyles which cause them.

While death from coronary heart disease has steadily decreased for more than 2 decades in many countries, no one knows whether the severity of coronary atherosclerosis, myocardial involvement, or the involvement of the conduction system, nerves, and ganglia of the heart in this period have decreased, increased, or stayed the same. In Olmsted County, Minnesota, a study of the coronary arteries by means of serial transverse sectioning at 5-mm intervals and grading of the severity of the lesions based on the degree of luminal narrowing did not detect a decrease in the severity of atherosclerotic involvement, which could explain the declining mortality from coronary heart disease.[56] In Hong Kong, the mortality due to coronary heart disease remained relatively low, but the severity of coronary atherosclerosis was comparable with that in western populations.[13] We did not find in the available literature a pathological investigation able to demonstrate that the decline in coronary heart disease mortality was associated with a diminution in the degree of luminal narrowing produced by stenotic or obstructive atherosclerotic plaques and thrombi.

In summary, a number of fundamental questions related to secular trends of coronary atherosclerosis and coronary heart disease remain to be answered. Coronary atherosclerosis, which has existed for thousands of years in man and the animal kingdom, has a very peculiar distribution. By the end of this century, the clinical manifestations induced by coronary atherosclerosis will remain virtually unknown in parts of Asia, Africa, and South America. The mortality rate in Europe continues to show geographic variations from north to south, more than three times the myocardial clinical manifestations induced by coronary atherosclerosis existing in Finland than in Spain, in Sweden than in Greece, and in Scotland than in Portugal. Superposed on this peculiar geographic distribution, the mortality rates decline, increase, or remain unchanged.

We hope that a more coherent picture on the relationship between risk factors and coronary heart disease and on the secular trends of coronary atherosclerosis will be given by the

MONICA Project (Monitoring the Trends and Determinants in Cardiovascular Disease), a multinational collaborative research project launched by the WHO.[57] It represents a valuable attempt to measure over a 10-year period both mortality and morbidity due to coronary heart disease, as well as changes in risk factors, lifestyles, and medical care in defined study populations.[58] This Project could give an adequate answer as to whether coronary heart disease mortality rates are declining because of secular trends, changes in diet and lifestyle, specific medical and surgical interventions, or because of factors that remain unidentified. An intriguing problem is the methodology, especially that used by pathologists. It will be difficult to accept that a 10 to 15% reduction in the coronary intimal surface covered with heterogeneous changes called fatty streaks, or with raised lesions which are difficult to correlate to impairment of coronary blood flow, as well as a 10 to 15% reduction in the lesion size or in its obstructive character would represent a solid proof of: significant changes in secular trends, efficacy of risk factor reduction, and improvement of specific medical care. An area of major deficiency continues to be our methods to examine the coronary arteries and this problem was largely discussed in Chapter 2.

B. Silent Myocardial Ischemia

The existence of silent myocardial ischemia was reported 3 decades ago, but we still lack adequate data on the percentage of cases in various age, ethnic, and geographic location groups; we also lack adequate data on sex and race differences and on the influence of genetic and environmental factors, particularly risk factors for coronary heart disease. An intriguing unanswered question is how silent myocardial ischemia relates to prognosis and long-term outcome of coronary heart disease. Certain investigators think that the presence, frequency, and magnitude of silent ischemic episodes could account for the important variability of myocardial clinical manifestations induced by coronary atherosclerosis. "What seems clear is that ischemia, whether silent of symptomatic, at whatever stage, renders individuals at much higher risk for cardiac events than the population at large."[59]

In certain ambulatory ECG studies, around 75% of ischemia cases were clinically silent.[60,61] Also of note is the observation that up to 50% of patients feel no pain before the first myocardial infarction. In the Framingham study more than 25% of myocardial infarctions were discovered only through the appearance of new diagnostic evidence during routine biennial ECG examination. Of these unrecognized myocardial infarctions, almost half were silent and the other caused atypical symptoms.[62] The proportion of all myocardial infarctions that were unrecognized was higher in women and in older men. In subjects with no preexisting myocardial clinical manifestations, the proportion of myocardial infarctions that were not recognized clinically increased with the severity of arterial hypertension: from 18.5% among normotensive men to 30 to 40% among men with definite arterial hypertension; for women this proportion increased from 27.8 to 44.6%.[63]

Many studies performed during the last decade used continuous ECG monitoring in patients with both unstable and stable angina pectoris. These investigations showed a high incidence of transient, ischemic-like ST-segment changes occurring without anginal pain. Some of these ECG changes were associated by impairment of left ventricular perfusion and function similar to that recorded during anginal pain.

Some investigators believe that the end stages of the natural history of coronary atherosclerosis manifested as angina pectoris, myocardial ischemia, and severe arrhythmias followed by sudden cardiac death always include a "transition period" during which myocardial ischemia remains silent. Moreover, it is tempting to presume that silent myocardial ischemia exists in all cases with severe stenotic plaques not bypassed by adequate collateral vessels. In addition, even in the absence of these severe stenotic plaques, individuals with angiographically documented spasm have been reported to have silent myocardial ischemia.

In essence, many cases of silent myocardial ischemia exist among apparently healthy

subjects. Gradually, cardiologists are becoming increasingly involved with these apparently healthy subjects who, on close investigation, show signs of silent myocardial ischemia, documented by ECG abnormalities and thallium myocardial perfusion defects. The association of coronary arteriography with other procedures may lead to a better detection of silent myocardial ischemia. In particular, noninvasive techniques are widely used for this detection, but all methods have severe diagnostic limitations. To reduce this source of error, many investigators use ECG, exercise tests, Holter monitoring, measurement of hemodynamic parameters, thallium scintigraphy, radionuclide angiocardiography, contrast angiography, and other methods. Since these complex investigations are difficult to perform, time consuming, and very expensive, such results are rarely published in the available literature.

According to certain subjective estimations, in the U.S. there may be 4 to 5 million cases with silent myocardial ischemia, including approximately 50,000 asymptomatic patients who have had myocardial infarction. There may also be 1 or 2 million asymptomatic individuals with no past history of myocardial infarction or angina pectoris.[64,65] Since 5 to 6 million patients are treated annually for coronary heart disease in the U.S., it might be presumed that for every three persons treated, another person with perhaps equally severe coronary atherosclerotic involvement is not being treated since his myocardial ischemia is silent. According to other estimations, silent myocardial ischemia would represent up to 10% of changes which may lead to myocardial clinical manifestations.[66,67] According to other views, the end stages of the natural history of coronary atherosclerosis, manifested as angina pectoris, myocardial infarction, severe arrhythmias, and sudden cardiac death, always include a succession of silent and symptomatic myocardial ischemia; only the symptomatic periods are usually recorded. Finally, there are also views which assume that silent myocardial ischemia appears to be more common than symptomatic ischemia and that all treatments directed at eliminating ventricular dysfunction must be first directed at silent ischemia. According to these views, silent ischemia should be treated as aggressively as symptomatic ischemia until additional data are available.[59] The fact that silent episodes are at least as common, if not more common, than symptomatic episodes and are present in almost all cases with severe atherosclerotic involvement of the coronary arteries is an important challenge for epidemiologists and cardiologists. It is possible that silent ischemic episodes contribute to a significant proportion of sudden cardiac deaths and myocardial infarctions year by year, but are usually overlooked since they are painless. It is also possible that development of ischemic cardiomyopathy without definite infarction could relate to silent myocardial ischemia. As a matter of fact, patients with silent myocardial ischemia may also mean patients with asymptomatic disturbances of left ventricular contraction, conduction defects, inadequate development of collateral vessels, etc. Angina may occur without systolic pump failure; on the other hand, systolic pump failure may develop without anginal pain during the transient stage of silent myocardial ischemia.

At present, it is safe to say (if unfortunately) that certain apparently healthy subjects walking in the streets or working in the factories and laboratories are, in fact, patients with silent myocardial ischemia. These patients seem to be at high risk to present in the coming years myocardial infarction, severe arrhythmias, and sudden cardiac death.

Once thought to be an unusual phenomenon largely confined to anecdotal reports, silent myocardial ischemia is now recognized to be a common occurrence in middle-aged and elderly people. With the widespread use of improved ambulatory electrocardiographic monitoring and such methods as continuous monitoring with telephonic transmission, it might be possible to investigate more adequately the incidence, pathogenesis, and in particular, the natural history of silent myocardial ischemia.

The available data suggest that silent myocardial ischemia would include:[68] (1) cases with significant coronary atherosclerotic involvement at angiography or post-mortem examination, without history of angina pectoris, myocardial infarction, arrhythmias, or congestive heart

failure and (2) cases asymptomatic during some episodes of myocardial ischemia, but not during others and asymptomatic following myocardial infarction.

An intriguing and still unanswered question is if these individuals with silent myocardial ischemia are, or are not, representative for those patients with coronary heart disease, whose first clinical manifestation will be ventricular fibrillation followed by sudden cardiac death? There is a pressing need to have more adequate and abundant data on the incidence of persons with silent myocardial ischemia who survive ventricular fibrillation, who experience clinically overt myocardial infarction, and who will present with congestive heart failure.

The view that the appearance of chest pain represents the onset of myocardial ischemia and of myocardial clinical manifestations induced by coronary atherosclerosis is one of the many dogmas which have dominated our thought. A sharp delineation was traced between asymptomatic and symptomatic cases and many epidemiologic and cardiologic studies are based on this unrealistic separation of these two groups. An effort has also been made by pathologists to detect arterial wall changes which appear in patients with symptomatic coronary heart disease and are not present in asymptomatic ones. Extensive involvement of the coronary arterial tree by stenotic or even occlusive atherosclerotic plaques is compatible with a normal lifestyle; a middle-aged adult considered to be a healthy subject may present on post-mortem examination following a traffic accident an atherosclerotic involvement of the coronary arteries as severe as that of patients who died of myocardial infarction or sudden cardiac death.[69,70]

Pathologists offered a very important scientific basis to demonstrate the existence of slient myocardial ischemia in apparently healthy subjects and also in asymptomatic patients with coronary heart disease. One can survive in the face of severe and extensive multivessel involvement by stenotic and obstructive atherosclerotic plaques if an adequate development of collateral vessels exists. This adequate or inadequate development of collateral blood flow has been deeply implicated in the onset or absence of chest pain.[71] Other mechanisms involved seem to be the extent and topography of the myocardial ischemic region, the reversibility or irreversibility of lesions, and especially, the pain threshold.

According to certain opinions,[72] from a pathophysiological point of view, an alarm system from the heart does not exist. Evidence is rapidly accumulating that during angioplasty, angioscopy, and other procedures, pain does not appear. This is in agreement with the difficulty in demonstrating the presence of cardiac nociceptors and with the results of experiments which showed that it is possible to produce a massive excitation of afferent sympathetic fibers of the left ventricle without eliciting pain. All the evidence available at present indicates that chest pain appears to be an insensitive indicator of transient myocardial ischemia and that physicians do not try to relieve all episodes of such transient myocardial ischemia, but only those which occur as angina pectoris.[73] It can be said that anginal pain represents only a portion of total ischemic episodes in patients with severe atherosclerotic involvement of the coronary arteries. This is in agreement with an increasing number of reports suggesting that myocardial ischemia may occur without any angina pectoris pain. There are many patients who never suffer angina in spite of repeated episodes of ischemia. Even epidemiologic studies which use anginal pain to delineate the end stage of the natural history of coronary atherosclerosis often include data on myocardial infarctions and sudden cardiac deaths which were not preceded by anginal pain. Therefore, it appears necessary to find and use additional criteria to detect myocardial ischemia.

There may be a debate over whether the presence of a defective anginal warning system in certain subjects or population groups implies a worse prognosis. Interindividual differences in sensitivity to pain may be related to variations of endorphine mechanisms, since varying concentrations of endorphines may represent the neurochemical substrate responsible for interindividual differences in pain sensitivity. Endorphines are endogenous opiates that have an effect similar to that of morphine and their level may vary in plasma and cerebrospinal

fluid, as well as in the intersynaptic area. This individual variation could explain why some patients show a hyposensitivity to pain and higher pain thresholds were detected in patients with silent myocardial ischemia. It is tempting to presume that asymptomatic myocardial ischemia may represent an early stage of myocardial clinical manifestation induced by coronary atherosclerosis in all subjects with a hyposensitivity to pain and implicitly a defective anginal warning system.

Pain impulses are carried preponderantly in sympathetic nerve fibers originating in the perivascular and adventitial networks, through the cardiac nerves to the paravertebral chain of cervical and thoracic ganglia, through the spinothalamic tract of the spinal cord, and finally to the posterolateral and ventral nuclei of the thalamus. Three main causes might be involved in giving rise to silent ischemia: (1) the most important seems to be a failure to release adequate amounts of the mediators of pain, (2) nervous system disease, and (3) alterations of thalamus which become unable to appreciate the painful stimuli as pain. The observations that many apparently healthy subjects have silent myocardial ischemia support only the first suggested pathogenetic mechanism related to release of mediators of pain.

It is not possible, however, to offer a satisfactory explanation, based on the above-mentioned suggestion, as to why a more rapid passage from asymptomatic to symptomatic stages of myocardial ischemia is recorded: in males than in females but only in the white race, in industrialized than in underdeveloped countries, in Finland than in Norway, in eastern than in western regions of Finland, etc. In individual cases the passage from asymptomatic to symptomatic myocardial ischemia is very rapid during the natural history of coronary atherosclerosis. These are patients with peculiar origins and branching patterns of the coronary arteries, with Marfan syndrome, familial hypercholesterolemia, Kawasaki disease, etc. In other cases coronary atherosclerosis remains asymptomatic in spite of multiple vessel involvement with severe stenotic lesions and certain of these cases may exhibit silent myocardial ischemia.

The most significant factors relating nonischemic to ischemic stages during the natural history of coronary atherosclerosis seem to be

1. The number of major coronary arteries involved by stenotic plaques
2. The occlusive character of superimposed thromboses
3. The association of occlusive lesions with an inadequate development of collateral vessels
4. Conduction defects
5. Generalized impairment of the left ventricular functions
6. Peripheral embolization of intramural thrombi

Traditionally, the goal of therapy in patients with coronary heart disease has been, first, to relieve pain, but the attempts to eliminate the pain are only a part of the effort to prevent myocardial ischemia. The available evidence indicates that the significance of silent myocardial ischemia is sometimes comparable to that of painful ischemia in terms of prognosis and severity of myocardial injury.[74] *The opinion that the appearance of chest pain represents the onset of myocardial ischemia must be abandoned,* just as many other dogmas in the field of the natural history of coronary atherosclerosis.

II. ANGINA PECTORIS

A. General Observations

Evidence that myocardial ischemia produced by coronary atherosclerosis is the cause of angina pectoris has been accumulated over two centuries.[75] Heberden's classical description of angina in 1772 detailed clinical characteristics, but provided no insight into pathogenesis.

Parry in 1799 and Burns in 1809 suggested that anginal pain is due to inadequate blood supply to the heart and many observations contributed to support this view, including:

1. The association of anginal pain with post-mortem presence of obstructive atherosclerotic lesions in the coronary arteries
2. The analogy of angina pectoris with intermitent claudication in terms of pathologic findings, precipitating factors, and clinical evolution
3. The association of angina pectoris with total coronary occlusion, able to give rise to myocardial infarction
4. The production of angina in patients with coronary narrowings by reducing oxygen supply, or by increasing oxygen demand with exercise
5. The production of lactate in the coronary arterial bed during the period of anginal pain
6. The association of anginal pain with cellular changes produced by an inadequate supply, such as depletion of high energy phosphates, shift from aerobic to anaerobic metabolism, decrease in the contractile function

A suggestion has been made on the existence of mixed ischemic subsets. They would include:[76] silent myocardial ischemia, unstable angina pectoris, mixed angina pectoris, myocardial infarction, and sudden cardiac death. Chest pain is absent in silent myocardial ischemia and present in angina pectoris; ST-segment changes on ECG, left ventricular wall motion abnormalities, arrhythmogenic potentials, and coronary stenotic plaques are present in all subsets.

Simply stated, we must rearrange our thoughts and accept the view that the borderline between coronary atherosclerosis with and without myocardial clinical manifestations continues to be ill defined. All results based on anginal pain to differentiate cases with and without coronary heart disease should be interpreted with caution. If the group of noncoronary heart disease individuals includes many cases with silent myocardial ischemia, the results may lead to erroneous conclusions.

The material presented in the previous section tried to demonstrate that ischemia is broader in scope than to be simply equated with angina. The silent episode can evolve to a painful stage, can remain silent, or terminate as silent myocardial infarction or sudden death. The painful stage may also progress to myocardial infarction or sudden death.

In many works, an erroneous assumption continues to persist, namely that chest pain represents the onset of myocardial ischemia. Recent research findings are altering this concept, since pain is frequently a late symptom and late phenomenon relative to the onset of myocardial ischemia. An unfortunate assumption is that anginal pain is a symptom of coronary atherosclerosis; it is interesting to note that 40 to 70% of cases with idiopathic hypertrophic cardiomyopathy experience anginal pain.

Each year approximately 1% of men in industrialized countries between the ages of 30 and 60 years experience anginal pain and other symptoms of coronary heart disease for the first time.[77,78] Of these cases which pass from asymptomatic to symptomatic myocardial ischemia, approximately 42% present anginal pain as a prodrome to myocardial infarction occurring as the first clinical manifestation of coronary heart disease. A smaller proportion of subjects with anginal pain are also at risk for sudden cardiac death.

In the context of coronary heart disease angina pectoris appears to represent only a clinical syndrome, one of the possible manifestations of acute, transient myocardial ischemia. This ischemia may be asymptomatic, or accompanied by dyspnea, or by rhythm disturbances associated with various electrocardiographic changes. Angina per se does not seem to be an obvious marker of greater severity of ischemia, or of the risk of dangerous arrhythmias. On the other hand, it is a not-infrequent prodrome of an acute myocardial infarction. In a study carried out in Bucharest, 23.5% of cases with unstable angina developed myocardial in-

farction or died suddenly, and in 58.8% of patients the angina became stable; moreover, in 17.7% it even disappeared.[79] One cannot predict whether in a given patient the symptoms will reflect stable, exercise-induced ischemic cardiac pain, or whether symptoms will be progressive, are crescendo in nature, and thus unstable. If the results of many retrospective investigations are taken into consideration, they suggest that myocardial infarction occurred in about 30% of cases during 2 to 6 months of follow-up, whereas sudden cardiac death did not appear to be a major risk.

Two broad classes of angina pectoris are usually presented in the available literature, related to two fundamentally different pathogenetic mechanisms:

1. **Classical** angina pectoris, in which stenotic or occlusive atherosclerotic plaques present in the major coronary arteries, prevent the blood flow from increasing in response to myocardial demands
2. **Variant** angina pectoris, in which the reduction in blood flow is related to changes in coronary artery tone

These two broad classes are only a schematic simplification and the term mixed angina pectoris has been proposed in addition for patients with episodes of angina caused both by an increase in oxygen demand and by transient impairment of blood supply.[80] The introduction of this last concept is related to the fact that the same patient could experience angina both as a result of an excessive increase in myocardial demand (secondary angina due to stenotic atherosclerotic plaques) and as a result of the transient impairment of coronary blood flow (primary angina due to coronary vasospasm). The complexity of factors and mechanisms which may lead to anginal pain could explain why this clinical syndrome became the main source of errors in cardiologic diagnoses.[81]

The occurrence of anginal pain is considered to indicate an acute imbalance between myocardial demands and coronary supply. The primary determinants of this greater myocardial oxygen demand are the heart rate, myocardial contractility, and ventricular tension at the onset of the systolic contraction.

Because left ventricular tension varies directly in proportion to intraventricular volume and pressure, an increase in either will augment wall tension and thereby will increase MVO_2 (myocardial oxygen consumption calculated as the product of coronary blood flow per minute and the coronary arteriovenous difference). A five- or sixfold increase in MVO_2 can be produced during isovolumetric pressure development, suggesting that the load against which the heart muscle builds up tension prior to ejection is a major component of MVO_2 requirement. It is not possible to discuss here the complexity of the regulation of myocardial oxygen delivery which appears to depend on multiple variables, but it is important to emphasize that some of these variables are amenable to pharmacologic interventions.

The occurrence of anginal pain as a result of catecholamine release shows that the excitement produced by this release is a very powerful cause of increased oxygen demand due to increasing blood pressure, heart rate, and ventricular contractility. In addition, exercise, smoking, digestion, mental and environmental stresses which produce transient changes in the coronary artery caliber, and even dreaming sleep (nocturnal angina) may be occasionally involved.

It has already been mentioned that we do not know why the clinical syndrome of angina does not develop in all cases with severe stenotic lesions in the major coronary arteries and main branch vessels, or in all individuals with coronary vasospasm, coronary microthrombosis, or other extracoronary conditions associated with myocardial ischemia. The problem appears further complicated by the finding of a very high incidence of stenotic atherosclerotic plaques in the general population, compared to the relative low incidence of angina pectoris. In Section I of this chapter, many data were presented which show that the heart may become

ischemic, but this ischemia may not be appreciated as chest pain. It is this latter group of cases that accounts for silent myocardial ischemia and even silent myocardial infarction in the absence of anginal pain. The result of the Framingham study revealed that in more than $^1/_3$ of cases, angina pectoris was spontaneously remitted in spite of severe stenotic plaques in the coronary arteries.[82] On the other hand, the same investigation showed that about $^1/_2$ of all deaths in persons with angina pectoris were sudden and occurred at about four times the rate of deaths in the general population.

Particular interest has been centered during the last 2 decades on so-called unstable angina, a clinical syndrome considered as an ischemic chest pain interposed between chronic stable angina pectoris and myocardial infarction. Unstable angina pectoris has been defined as the appearance of ischemic pain in a patient previously without this syndrome, or the development of more intense pain, often at rest, in a patient with previously well-established classical stable angina pectoris. Cases with stable angina may have a crescendo increase in symptoms, characterized by more severe pain of longer duration brought on by lesser degrees of exercise. In some cases, the syndrome of coronary spasm and unstable angina were produced by injection of ergonovine and reversed by administration of nitrates. In other patients, spontaneous reversible spasm has been documented arteriographically during bouts of pain in unstable patients, whereas scintigraphic studies have shown a massive transmural reduction of myocardial tracer uptake during attacks associated with ST-segment elevation.

A suggestion has been made that in patients with unstable angina circulating platelets become activated and adhere to exposed collagen at the site of advanced atherosclerotic plaques where a release reaction takes place. Particularly, thromboxane A_2 (TXA_2) seems to be able to produce platelet aggregation and coronary vasoconstriction; both favor the formation of platelet plugs which obstruct the coronary artery lumen and also disintegrate and produce emboli. Thus, increase in TXA_2 is suggested to initiate or sustain the syndrome of unstable angina pectoris, in which an imbalance between TXA and PGI_2 was suggested to exist.[84] This view is supported by the beneficial effect of aspirin in many patients with unstable angina, the evolution toward myocardial infarction, and sudden cardiac death being 8.6% in the group given aspirin and 17.0% in the other groups.[85] Small doses of aspirin per day are considered adequate for suppression of platelet cyclooxygenase activity and hence of TXA_2 production, whereas higher doses will increasingly suppress the potentially beneficial synthesis of PGI_2 in the vessel wall. Significant improvement was recorded in patients with unstable angina who were treated with an intravenous infusion of PGI_2.[86] These results suggest that after a delay during which TXA_2-mediated spasm and hypoxia may predominate, sufficient PGI_2, a potent vasodilator and inhibitor of platelet aggregation, may form to terminate the spasm and partially (at least) to clear the microcirculation overcrowded with platelet microemboli. In this manner, an episode of angina may end without evolving toward myocardial infarction or lethal arrhythmias. The frequent presence of mural thrombi able to disintegrate and to give rise to intramyocardial emboli in patients with unstable angina suggests that our rate of error in predicting angiographically the absence of organic disease based on the absence of visible stenotic lesions is rather high. The formation and disintegration of mural thrombi which are not visible angiographically must be taken into consideration in the pathogenesis of unstable angina, in addition to the left main coronary artery involvement, multivessel involvement, and myocardial contraction abnormalities. A certain role has also been attributed to products released by mast cells, an elevated number of mast cells being detected in coronary arteries containing both fresh and organized thrombi.[87] Histamine has been used successfully in a provocative test to induce spasm in patients with suspected unstable angina pectoris. The arachidonic acid products secreted by mast cells, prostaglandins, and leukotrienes may also be important modulators of coronary vascular tone. Note that at the site of the spasm of the left anterior descending artery the number of mast cells was higher than in any other site of the coronary arterial tree.[87] Histamine increased

rhythmic activity and tension even in isolated human epicardial coronary segments excised 3 to 5 hr after sudden death.[88] This ability of human coronary smooth muscle to contract spontaneously is considered to be the main cause of spontaneous coronary artery spasm and angina pectoris. In experiments on isolated epicardial coronary arteries of dogs and cats, these phasic spontaneous contractions could not be recorded.[88]

New onset of anginal pain or worsening of previously stable exertional anginal may also reflect the appearance and disintegration of thrombi produced by plaque rupture, this rupture usually determining the evolution of an unstable angina toward a myocardial infarction. A variety of local changes may occur which cannot be visualized on coronary angiography as intraplaque hemorrhage, embolization of disintegrated thrombi in the intramyocardial vessels, changes in platelet aggregability, or altered coronary responsiveness. Embolization of disintegrated thrombi may be followed by impairment of the coronary reserve and this change must be regarded as an important contributor in the pathogenesis of unstable angina pectoris. This impairment of coronary reserve is more important from the point of view of hemodynamic consequences in subjects without than in subjects with an adequate collateral circulation, and some investigators have even suggested that unstable angina pectoris may be related to a relative lack of intracoronary collaterals.[89,90]

According to other results, the nervous system is more or less involved in the pathogenetic mechanisms of unstable angina pectoris, increased heart rate, and/or blood pressure being recorded prior to the development of pain. This would indicate that an autonomically mediated sympathetic discharge, with subsequent increase in systemic vascular resistance and heart rate, was at work. A suggestion has been made that the metabolic consequences of ischemia could stimulate a chemoceptor to set off a reflex sympathetic discharge with secondary rises in blood pressure and heart rate. If this were so, it would imply a positive feedback mechanism: myocardial ischemia produces increased cardiac work by means of sympathetic reflexes which in turn would cause further ischemia. This feedback mechanism could eventually lead to irreversible myocardial ischemia with a resultant infarction area.[90]

On the basis of present knowledge, it seems unlikely that various forms of angina pectoris presented in the available literature and particularly variant angina or unstable angina can be considered well-delineated anatomoclinical entities. An eminent authority considers variant angina one aspect of a continuous spectrum of vasospastic myocardial ischemia.[91,92] Much remains to be learned about various subgroups, pathogenetic mechanisms, and methods of prevention. On the other hand, the use of the terms "stable" and "unstable" angina continues to preserve both clinical and prognostic significance and may serve as a guide to how carefully and intensively the patient should be treated. The available literature shows that one out of four or five patients with unstable angina exmined by an angiographer will present in the next days or months clinical symptoms of myocardial infarction. A variety of other terms are added by investigators and clinicians to characterize this peculiar condition: intermediate syndrome, pre-infarction syndrome, and acute coronary insufficiency, but the label unstable angina seems to be more widely accepted. Irrespective of the term used, it designates the clinical spectrum of a dynamic, yet reversible interplay of many of the factors involved in the complex pathogenesis of coronary heart disease. As has already been mentioned in this complex pathogenesis the variant or unstable forms of angina pectoris represent only particular manifestations of a continuous spectrum of vasospastic acute myocardial ischemia that can be recorded in practically any phase of the natural history of angina pectoris, myocardial infarction, or sudden cardiac death. Vasospastic myocardial ischemia may occur in the presence of an extremely variable extension and severity of coronary atherosclerosis and this supports the assumption that the natural history of coronary atherosclerosis is not superposable, but only partly intricated with that of the angina pectoris, myocardial infarction, and sudden cardiac death.

B. Anatomoclinical Correlations

It has been firmly established and largely accepted that *in angina pectoris a severe atherosclerotic involvement of the coronary arteries is almost universally present.*

Most arteriographic and post-mortem studies have confirmed the association of angina attacks with stenotic atherosclerotic plaques in the major coronary arteries and with spastic occlusion of diseased vessels. On the other hand, the comparison of the high incidence of severe coronary atherosclerotic involvement in the middle-aged adult population and the low incidence of anginal pain demonstrates that the presence of coronary stenotic plaques per se cannot be considered the only determinant of the clinical manifestations. This is consistent with the finding at necropsy of severe stenotic or even occlusive atherosclerotic plaques in the coronary arteries in a considerable number of apparently healthy persons dying of accidental causes.

Many pathologists have described in detail the extensive coronary atherosclerotic involvement and particularly these stenotic plaques present in angina patients. On the other hand, *no specific morphologic basis for angina has ever been established* and no particular anatomical feature has been consistently found to be associated with a history of long-standing angina pectoris.[93]

In clinically isolated angina pectoris, a necropsy study may record diffuse luminal narrowing of the major coronary arteries, but relatively little myocardial damage. In a special investigation, it was possible to demonstrate that the atherosclerotic plaques involved long segments of the coronary arterial wall and the degree of luminal narrowing was severe in all; the average number of coronary arteries narrowed more than 75% are the same in patients with stable and unstable angina.[94] Significant differences were detected in a comparative post-mortem analytical study on the severity of coronary arterial narrowing in patients with unstable angina pectoris and in control subjects of similar age and sex who died from noncardiac conditions. In patients with unstable angina pectoris 47% of vascular segments investigated were 76 to 100% narrowed in cross-sectional areas by atherosclerotic plaques, compared to only 1% in controls; also, 79% of vascular segments investigated in patients with unstable angina pectoris were 51 to 74% narrowed, as compared to 29% in the controls. The amount of severe (more than 75%) narrowing of the right coronary, left main, left anterior descending, and left circumflex arteries by atherosclerotic plaques was apparently similar. Of particular importance is the observation that $^3/_4$ of the length of the four major epicardial coronary arteries in the 22 patients studied was more than 50% narrowed in cross-sectional areas by atherosclerotic plaques.[95] This diffuse atherosclerotic involvement, which was also recorded in our studies, contrasts with the results obtained by the usual macroscopic grading system based on the extent of the intimal surface covered with fatty streaks and raised lesions. This method revealed in our patients with angina pectoris only 30% of the intimal surface involved by atherosclerotic lesions. Moreover, strict adherence to this protocol overlooks the presence of severe stenotic plaques, mural thrombi, and occlusive lesions in arteries such as posterior descending, first diagonal, first septal, left, and right marginals and in the vessels which supply the conduction system.

A mural thrombus developing in the proximal segment of the posterior descending or first diagonal arteries may be a source of peripheral embolization of intramyocardial vessels and a cause of impairment of coronary reserve of similar importance as a mural thrombus developing in the right coronary artery or left anterior descending artery. We have repeatedly mentioned in this book that it is not possible to offer a realistic anatomoclinical correlation in both asymptomatic and symptomatic myocardial ischemia without a complete post-mortem study of the whole coronary arterial bed.

Many angiographic observations suggest that the clinical severity of angina pectoris does not accurately reflect the degree of severity of coronary atherosclerosis: a severe disease may exist with an involvement of only one major coronary artery and a moderate angina

pectoris with an extensive atherosclerotic involvement of all major coronary arteries. In certain studies narrowings were found in about 75% of patients with unstable angina pectoris, as opposed to only 30% of a similar group, including patients with stable angina pectoris.[96] Such results would indicate that progression in the severity of coronary atherosclerotic plaques can contribute to the occurrence of unstable angina. Except for this progression of lesions, there is no pattern of coronary artery involvement by atherosclerotic lesions pathognomonic of a specific clinical syndrome manifested as angina pectoris. This conclusion, formulated 2 decades ago,[97] continues to be largely accepted. A second important conclusion which continues to persist is that the severity and extension of the lesions are not necessarily proportional to the length of the patient history. However, as has already been mentioned, certain results suggest that progression in the severity of coronary atherosclerotic plaques can contribute to the occurrence of unstable angina pectoris.[96] Patients with a recent onset of anginal pain have a high angiographic incidence of severe atherosclerotic involvement of only one major coronary artery; when a stable angina is replaced by a unstable one with a crescendo character, the severe involvement of all major coronary arteries is usually recorded.

A patient who is not operated upon runs, according to angiographic data, a 10%/year risk that a preexisting lesion will worsen and a 5%/year risk that a new stenotic lesion will appear.[98] Important from a therapeutic standpoint is that in about 50% of patients, at least one obstructed vessel is angiographically operable. The available literature suggests that about 25% of the patients with angina pectoris have critical stenosis in only one major coronary artery, more than 30% in two major coronary arteries, and more than 40% in three major coronary arteries. The real value of this critical stenosis was discussed elsewhere (Chapter 2).

The angiographic pattern of coronary atherosclerotic involvement in patients with angina pectoris was also correlated with electrocardiographic changes, especially the direction, magnitude, and location of ST-segment shifts being associated with the topography, size, and severity of coronary atherosclerotic plaques. According to certain results, in single vessel disease, the ST-segment tends to be elevated if the stenotic plaques develop in the right coronary artery and tends to be depressed if the plaque develops in the left circumflex artery; both changes up or down may appear if the left anterior descending artery is the vessel involved.[99] Alteration of the ST-segment is considered of major importance, particularly in variant angina, defined as angina pectoris associated with transient ST-elevation of the electrocardiogram during attacks, as opposed to classical angina, which is usually associated with ST-segment depression.[100] Many investigators believe that the location of the ST-segment elevation on the ECG may localize the region of myocardium involved and may help to predict the coronary artery in which a transient occlusion would occur.

In essence, both angiography and ECG are of particular importance for delineating the successive stages of the natural history of angina pectoris and for relating it to the natural history of coronary atherosclerosis. Important progress in this field was accomplished with the possibility of a second and third catheterization at variable intervals after the first study. Repeated coronary angiography revealed that about 20% of significantly blocked arteries worsened in 25 patients who were restudied after an average of 12 months of optimal medical treatment.[99] All obstructions greater than 90% at the first study progressed to total occlusion and in six out of seven patients who developed complete obstruction, a myocardial infarction appeared. Angiographic aspects suggesting regression of lesions were also recorded. It has become apparent from repeated coronary angiography that progression of lesions toward total vessel obstruction was sometimes associated with an important development of collateral vessels; in certain studies there were no significant changes in collaterals when complete obstruction did not ensue.[101] This occlusion was often associated with progressive development of myocardial infarction in the ischemic area, especially in patients with unstable angina pectoris. Arteriographic progression of lesions was also detected in patients whose

clinical status has not clearly worsened. In the state of the art, comparison of arteriograms from different subjects, or at different times in the same subject, may be of limited value for assessing severity, changes in severity, or functional significance of coronary artery stenoses.[102,103] There is general agreement that many difficulties appear in an attempt to distinguish adequately between 75 and 80% luminal narrowing, or even 80 and 90% stenoses. In addition to the degree of narrowing, many geometric characteristics of stenoses, such as length, absolute diameter, multiple lesions in series, and the eccentric or concentric character of plaques may be different in one view compared with another. Many studies related to the angiographic morphology and pathogenesis of unstable angina pectoris have emphasized the heterogeneity of aspects revealed. An attempt has been made to include these heterogeneous aspects into four types:[104] concentric stenoses; eccentric stenoses with asymmetric narrowing, smooth borders, and a broad neck; eccentric type with a narrow neck or irregular borders or both; and multiple irregular narrowing in series. The third type was significantly more frequently recorded in patients with unstable angina, whereas the first and second types were more frequently revealed in patients with stable angina. This frequency in patients with unstable angina of eccentric stenoses, asymmetrical, with a narrow neck or irregular borders or both, would reflect the existence of ruptured atherosclerotic plaques or partially occlusive thrombi or both.[104] This suggested aspect of ruptured plaque could be associated with the development of occlusive thrombi and acute progression of clinical events. Dissolution of such intraluminal filling defects with streptokinase in patients with unstable angina[105] may advocate for this suggestion. Such thrombi are usually located just distal to the coronary narrowing on angiography.

An intriguing question is whether the onset of unstable angina pectoris is associated only with a worsening of the preexisting lesions or also with a peripheral embolization from mural thrombi. The role of progression of coronary atherosclerotic plaques toward stenotic lesions as a main cause of the onset of unstable angina pectoris was demonstrated in many studies using repeated coronary angiography performed before and after the occurrence of anginal pain. As concerns the role of a peripheral embolization from mural thrombi, this remains more difficult to demonstrate. It is largely accepted that the occurrence of coronary mural thrombi in response to injury to the endothelium includes repeated cycles of build-up and disintegration of platelet aggregates. Many angiographic observations support the view that coronary thrombosis is not a static, but a dynamic lesion, being submitted to the action of factors which cause it to fragment or resolve. Two sequences of events were suggested to occur.[106] The first commences with plaque rupture which is followed by thrombosis and coronary vasospasm; the second commences with coronary vasospasm, followed by plaque rupture and thrombosis. According to certain results, when the stenosis was greater than 90% as determined histologically, plaque rupture was nearly always associated with occlusive thrombosis.[107]

Both occlusive and nonocclusive thrombi superposed on preexisting atherosclerotic plaques have a surface able to generate emboli and showering in the intramyocardial vessels of platelets aggregates may help to explain certain as yet unexplained severe arrhythmias and even sudden cardiac deaths. This intramyocardial dissemination of platelet aggregates may also help to explain the occurrence of myocardial ischemia manifested clinically as anginal pain. A coronary thrombus which builds up to a critical size, then breaks off, and its small fragments are carried downstream may be deeply involved in the pathogenesis of unstable angina. Whereas an occlusive thrombus is usually associated with a full thickness infarct, a mural thrombus may lead to an occlusive lesion followed by myocardial infarction, may occur associated with focal ischemic lesions of a variety of types which remain asymptomatic, or may give rise to angina pain, severe arrhythmias, and sudden cardiac death.[108] If unstable angina pectoris is produced by a dynamic formation and disintegration of intramural thrombi, then it is tempting to presume that myocardial infarction may occur in a patient with angina

pectoris when the thrombus becomes unable to disintegrate and appears totally occlusive. The additional role of coronary vasospasm in this complex sequence of events is possible in any stage of the respective sequence. If this dynamic view is accepted, the pathology of angina pectoris based only on the intimal surface of the coronary arteries involved with fatty streaks and raised lesions, or on the static aspects of luminal narrowing, requires reconsideration. According to this dynamic view the most important events seem to be the formation, disintegration, and reformation of thrombi and the repetition of this cycle. This would also indicate that the prevention of thrombus formation may represent the focus of our effforts to reduce the morbidity and mortality rates of coronary heart disease.

III. MYOCARDIAL INFARCTION

A. Myocardial Infarction Location as Related to Coronary Atherosclerotic Involvement

By definition, the great majority of pathologists, cardiologists, angiographers, and experimentalists consider infarction an area of myocardial necrosis due to cutting off the blood supply to the respective region.

If the human coronary arteries would have a unique and invariable type of anatomical branching pattern, as is erroneously presented in many books and journals and if all lesions able to occlude the vessel lumen would develop only in the proximal segment of the major coronary arteries which are opened longitudinally during autopsy, the relationship between myocardial infarction location and coronary atherosclerotic involvement would be very simple and easy to understand.

In the autopsy room, the observations made are by far more complex, since one can see:

- Complicated plaques which completely occlude a major coronary artery, whereas the myocardial area supplied by the respective vessel is apparently normal
- Myocardial infarction coexisting with an insignificant atherosclerotic involvement of the vessel supplying the infarction area
- An occlusive lesion only in a major coronary artery, associated with the presence of a circumferential infarction, in spite of the absence of severe stenotic lesions in the other major coronary arteries
- An apical infarction with an occlusive lesion only in the intermediate segment of the left anterior descending artery which is not opened during routine autopsy
- A lateral infarction with an occlusive lesion only in the proximal segment of the left marginal branch of the left circumflex artery which is not opened during routine autopsy
- A septal infarction with an occlusive lesion only in the proximal segment of the first and/or second septal branch, which are not opened during routine autopsy
- An anterior infarction with an occlusive lesion only in the proximal segment of the first and/or second diagonal branch, which are not opened during routine autopsy
- A posterior infarction with an occlusive lesion only in the proximal segment of the posterior descending branch of the right coronary artery (or of the left circumflex artery), which is also not opened during routine autopsy
- Anterior infarction associated with an occlusive lesion in the right coronary artery or in the left circumflex artery
- Lateral infarction associated with an occlusive lesion in the right coronary artery or left anterior descending artery
- Posterior infarction associated with an occlusive lesion in the left anterior descending artery
- Many other aspects of paradoxical coronary thrombosis, infarction at a distance, ectopic infarction, etc. — all these terms refer to the presence of an occlusive lesion in a major coronary artery, without an acute myocardial infarction in the terminal region

of the vessel involved; this is associated with the existence of a large area of myocardial necrosis in the region supplied by an artery which is not actually occluded by an atherosclerotic lesion

- Occlusive lesions in the atrioventricular node artery associated with small areas of necrosis in the intraventricular portion of the conduction system in myocardial infarction associated clinically with permanent bundle branch block

The complexity of these findings furnished by a meticulous gross inspection and light microscopic investigation of the whole coronary arterial tree is augmented by the variability of collateral vessel development, particularly between the right coronary artery and the left anterior descending or left circumflex arteries. These collateral vessels cannot be detected on post-mortem examination without special methods.

In many cases with the so-called paradoxical coronary thrombosis or infarction at a distance, as well as in patients with circumferential myocardial infarctions, we were often unable to correctly relate the age of the coronary occluding thrombus with that of the areas of myocardial necrosis. A real danger exists for over- or underinterpretation, since both thrombi and infarction area go through successive stages of evolution which cannot be clearly revealed by static histopathologic examination. In addition, in circumferential infarction, we often overlooked the existence of zonal (subendocardial) area of necrosis, or of laminar infarction. These small areas of myocardial necrosis are sometimes associated with intraventricular mural thrombi.

There are several ways to relate the topography of myocardial infarction area to the topography of stenotic or occlusive atherosclerotic plaques. One of these ways is to divide the left ventricle into radial segments; attempts were also made to relate the topography of myocardial infarctions with various electrocardiographic and angiographic patterns. To diminish confusion between ECG patterns and anatomic locations of myocardial infarctions, utilization of a common terminology would appear necessary, since certain terms used in ECG location are usually not employed to designate the location found at autopsy.[109]

Schematically, coronary atherosclerotic lesions exhibiting a stenotic or occlusive character might be involved in the onset of myocardial infarctions in six major topographic areas of the heart.

Area I: The portion of the left ventricular wall extending from the interventricular septum to the anterolateral papillary muscle or the so-called anterior region of the left ventricle. The necrotic areas developed in this region are designated anterior infarcts and the atherosclerotic lesions involved are usually present in the left anterior descending artery (proximal and/or intermediate segments) and first diagonal artery (proximal segment).

Area II: The portion of the left ventricular wall extending from the interventricular septum to the lateral margin of the posterolateral papillary muscle of the so-called posterior region. The necrotic areas developed in this region are designated posterior infarcts and the atherosclerotic lesions involved are usually present in the right coronary artery and its posterior descending branch, or in the left circumflex artery and its posterior descending branch. In the right coronary artery, the obstructive lesions are often located in the intermediate segment, between the branching point of the right marginal and posterior descending vessels.

Area III: The left ventricle between the two papillary muscles or the so-called lateral wall, in which lateral infarcts develop. The occlusive lesions are usually present in the left circumflex artery and/or in its left marginal branch, sometimes also in the left anterior descending and diagonal arteries.

Area IV: The interventricular septum, supplied by the septal branches of the left anterior descending artery and of the posterior descending artery. The necrotic areas developed in this region give rise to septal infarctions. The occlusive lesions may be present in the left anterior descending artery, first septal artery, posterior descending artery and right coronary

artery or left circumflex artery; they are sometimes also present in the atrioventricular node artery.

Area V: The left ventricular apex, supplied by the left anterior descending artery; the necrotic areas developed in this region give rise to apical infarctions.

Area VI: The right ventricle, its necrotic areas being usually associated with other zones of myocardial infarction belonging to the left ventricle.

Of note are also those eight sites proposed for the left ventricle:[110] anteroseptal, anterolateral, anterobasal, inferior, inferobasal, lateral, septal, and apical. Overlapping among these sites can be detected in many investigations.

The difficulties encountered by the pathologist in relating the topography of the infarcted area with the topography of the occlusive lesion are augmented when he detects two, three, or more healed infarcts in the same heart; this was recorded in certain investigations in more than $^1/_2$ of the patients studied. Moreover, one of five patients with healed infarcts may exhibit an acute infarction. These difficulties are, in addition, augmented by the fact that in nearly $^1/_2$ of the patients one could find at post-mortem examination only a subendocardial infarction. Unfortunately, data offered by ECG appear very reliable in distinguishing between infarctions involving the anterior wall from those involving the posterior wall, but less reliable in separating other particular topographic areas of myocardial necrosis. Thus, for instance, ECG data are unable to delineate isolated anterior wall infarcts from anterolateral or anteroseptal, or posterolateral from posteroseptal, or in detecting posterior wall infarction in patients who also have anterior wall infarction. ECG seems to be a poor predictor whether an infarct involved the basal or the apical portion of the left ventricle, whether a healed infarct is subendocardial or transmural in location, or in predicting the extent of the infarct.[109] Patients with posterior infarction are often poorly diagnosed by standard ECG criteria and many difficulties may also appear when ECG is used to reveal the presence of myocardial infarction in the interventricular septum and lateral wall of the left ventricle.[111]

In our material, myocardial infarctions were present in 41% with an anterior location, 23% with a posterior location, 15% lateral, and 15% septal.

The anterior infarcts extended more than 4 cm in length and exhibited an irregular outline. The largest infarcts were constantly associated with an occlusive thrombus at 1 to 3 cm from the point of origin of the left anterior descending artery and in $^1/_2$ of patients in the proximal segment of the first diagonal artery. Some infarction areas also extended lateral, basal, apical, and septal. More than $^1/_3$ of the patients investigated had two or three healed infarcts. The precise ECG location of the healed myocardial infarction was confirmed at necropsy in more than 70% of cases. About $^1/_2$ of the anterior infarctions showed areas in which the lesion was subendothelial, alternating with transmural aspects, and about $^2/_3$ did not have involvement of the anterolateral papillary muscle. When the anterior infarction area extended into the interventricular septum, involvement of the conduction system may appear. The patients showed various types of bundle branch blocks, some of which were transient in nature. In only 3 of 18 cases with interventricular septum extension of the anterior infarction area were we able to detect small areas of necrosis of the conduction system. The prevalent change appeared as hydropic swelling of cells of the proximal bundle branches, a finding also mentioned in other studies.[112] This hydropic cell swelling, associated with interstitial edema, may lead to cell death, but cannot be considered by itself an irreversible change. The prognosis of many anterior infarctions in our studies was related to the effects of this intraseptal extension associated with the involvement of the conduction system.

The posterior infarctions were usually associated with thrombosis or severe stenosis of the proximal and/or intermediate segments of the right coronary artery; they were also associated with occlusive or obstructive lesions in the right marginal and posterior descending branches of the right coronary artery (or in the posterior descending branch of the left circumflex artery). The great majority of these infarcts extended into the posterior part of

the interventricular septum and in certain patients the necrotic lesion exhibited a circumferential aspect. In such cases, a severe atherosclerotic involvement was revealed in all major coronary arteries and in their main branch vessels. This circumferential form may involve 70 to 75% of the left ventricle mass and its occurrence was constantly associated with cardiogenic shock. Posterior infarction, in our material, almost inexorably affected the posteromedial papillary muscles. One out of four patients with posterior transmural myocardial infarctions of the left ventricle also had extensions involving the posterior wall of the right ventricle. In fact, posterior myocardial infarction is rarely, if ever, limited to the posterior wall of the left ventricle. As already mentioned, one might presume involvement of the posteromedial papillary muscle from knowledge that the myocardial infarction is posterior. The posterior papillary muscle has been reported to be affected more often than the anterior muscle, since the anterior papillary muscle has a more complex vascular supply, whereas the posterior papillary muscle is supplied only by the right coronary artery or the left circumflex artery, being very susceptible to ischemic events due to the absence of collateral vessels.

Infarction of the interventricular septum is associated with occlusive or severe stenotic plaques in the anterior descending and first septal arteries, or right coronary artery and its posterior descending branch, or left circumflex artery and its posterior descending branch. This particular location of myocardial necrotic areas is frequently associated with severe conduction disorders. The nature of conduction disturbances after interventricular septal infarction depends on the area affected, a posterior extension involving the main trunk of the bundle of His, the anterior extension accompanying bundle branches either at their bifurcation or in the muscular mass, followed by various types of blocks. When infarction extension is posterior, these septal lesions are associated with incomplete and transitory blocks; when infarction extension is anterior, the necrotic area is associated with complete and persistent blocks.

We could not detect any particular features related to the development of lateral and apical myocardial infarctions. We did not find in our material atrial infarctions, whereas the incidence of right ventricular infarction was less than 15%, and, as already noted, the right ventricular infarction was usually associated with concomitant involvement of the left ventricle. Differences in blood pressure, blood flow, and blood supply were proposed to explain the rare incidence of right ventricle infarction and cases are reported in the available literature of pure right ventricle infarction, associated with severe right ventricular hypertrophy and pulmonary arterial hypertension. In the pig, occlusion of the right coronary artery was usually followed by infarction of the posterior wall of the left ventricle and interventricular septum, but not of the right ventricle. If the occlusion of the right coronary artery was preceded by right ventricular hypertrophy and pulmonary hypertension, infarction of the right ventricle followed the occlusion.[113] The occurrence of a pure infarction of the right ventricle in man in the absence of left ventricle infarction, right ventricle hypertrophy, and pulmonary arterial hypertension may also be related to a deficiency of collateral vessels which connect the vascular bed of the left anterior descending artery and of the right coronary artery. The right coronary artery usually supplies the entire right ventricle except for the anterior margin supplied by the left anterior descending artery, or the conus artery. Pure infarctions of the right ventricle seems often to be associated with severe stenoses or occlusive lesions in both left anterior descending and right coronary arteries.[114] Because the left anterior descending artery is the primary source of collateral blood flow for the right coronary artery, collateral blood flow to the right ventricle may have been impeded in these particular cases. A peculiar high incidence of right ventricular infarction was recorded in certain coronary care units.[114,155] An additional particular observation is that fatal combined right and left ventricular infarction is associated with an unusually large incidence of acute coronary arterial lesions.[116] These acute lesions of both occlusive and nonocclusive character appeared distributed throughout

the whole coronary arterial tree and their onset might lead to an important decrease in collateral flow and to marked aggravation of cell damage in areas with persistent ischemia.

B. Which Comes First: Coronary Thrombosis or Myocardial Infarction?

Since 1881, the etiopathogenetic interpretation of myocardial infarction in man has been based on Conheim's concept that the coronary vessels are end vessels. Although this concept was not supported by the results of many studies on the anatomy, physiology, and pathophysiology of the coronary arteries, it still persists and appears prevalent in some schools of thought. In fact, all investigators which center their methodology and interpretation on a very exact estimation of the degree of coronary narrowing are the partisans of the Conheim concept. Based on this concept, a critical degree of luminal narrowing that invariably produces myocardial ischemia and even coronary heart disease has been defined by certain investigators as at least 75% stenosis. These views deliberately overlook the elementary truth that whenever adequate collateral vessels exist, evaluation of the ischemic significance of a stenotic lesion is per se impossible.

In 1919, a new, important step in the field of anatomoclinical correlations concerning the natural history of coronary atherosclerosis was made. The first cases of thrombotic occlusion of atherosclerotic coronary arteries associated with myocardial infarction were more deeply investigated.[117] The conclusion of this investigation was that coronary thrombosis comes first and produces myocardial necrosis, which leads to particular clinical manifestations and specific ECG changes. This cause-effect relationship between coronary thrombosis and myocardial infarction became so largely accepted that many cardiologists used the terms coronary thrombosis and myocardial infarction as synonymous. This also led to the largely accepted definition of myocardial infarction: ischemic myocardial necrosis due to coronary occlusion. This definition maintains that obstruction anywhere in the major coronary arteries will cause myocardial infarction. This was also demonstrated in many experimental models in which coronary blood flow was suddenly interrupted. There is also some evidence to indicate that an occlusive coronary artery lesion takes a variable amount of time from the onset of the respective plaque to complete occlusion. During such time this obstructive lesion may be a source of emboli, shed from the site, lodged in the small intramyocardial vessels, and causing myocardial ischemia in much the same way as transient ischemic attacks follow ulceration of an atherosclerotic plaque located in the carotid sinus.[118] This hypothesis is an attempt to explain the variable clinical features of myocardial infarction, particularly the intermittent progression or recurrence of the infarct, as well as the patchy nature of some infarcts.

The view that myocardial infarction is ischemic myocardial necrosis due to coronary occlusion could not substantiate the unexpected increase in the second half of this century in the incidence of myocardial infarctions without a corresponding augmentation in the number of coronary occlusive thrombi, as revealed in post-mortem studies. This raises the question of whether other mechanisms not associated to coronary atherosclerosis play a significant role in inducing myocardial infarction. The large use of in vivo coronary angiography also offered many examples which demonstrated that a coronary obstruction is not a prerequisite for the occurrence of myocardial infarction. All these results led to a reconsideration of a view mentioned 50 years ago, according to which coronary thrombosis is but a secondary event, the result and not the cause of a myocardial infarction.[119] This apparently peculiar and unfamiliar concept was progressively supported by the extreme variability in the incidence of coronary occlusive thrombi found in various samples from patients who died of myocardial infarction. In essence, ischemic necrosis or infarction of the myocardium can logically be attributed to impaired blood supply, but the frequency with which thrombotic occlusion of coronary arteries has been found in fatal myocardial infarction varies widely.

The role of thrombosis as the main initiating mechanism in the development of myocardial infarction was also questioned by the results of studies emphasizing the particular importance of the length of survival. With a survival of 1 hr the incidence of coronary occlusive thrombi was 16%, after 1 to 8 hr, 35%, and after more than 24 hr, 54%.[120,121] The rising frequency of a demonstrable occlusive thrombus with longer survival suggests that thrombosis was secondary to an as yet unexplained myocardial ischemia in patients with advanced coronary atherosclerosis. Also of note is the microscopic evidence that the coronary occlusive thrombosis in some instances might be more recent than the corresponding myocardial necrosis.[122,123] In certain samples, the infarct appeared to be older than the thrombus in approximately $^1/_3$ of cases of acute myocardial infarction with an associated coronary artery thrombosis.[69,70,124,125] Thrombotic coronary artery occlusion was less common in patients dying of sudden cardiac ischemia where the full clinical picture had not developed than in a series of hospital patients dying of myocardial infarction or ischemic coronary heart disease.[126] Attempts were also made to further clarify the time relationship between coronary occlusive thrombi and myocardial infarctions by means of incorporation of labeled fibrinogen into the developing thrombus.[127] The results favor the notion that the coronary occlusive thrombi in fact might be a secondary event in a myocardial infarction. The reports which exist on patients who develop myocardial infarction after bypass surgery in the presence of patent grafts also support the assumption that myocardial infarction comes first. In such cases, the passage from a stenotic to an obstructive lesion and even to a total occlusion might have an insignificant effect on the coronary blood flow.[124,125] Finally, in a study carried out on 107 patients with fatal acute myocardial ischemia, the frequency of coronary thrombosis was less in patients dying within 6 hr of onset than in those surviving longer.[128] Thrombi were found in none of the 9 patients with subendocardial necrosis only and in 8% of the 24 who died suddenly. These results would indicate that coronary arterial thrombosis was secondary and that a period of survival after the onset of infarction is the usual requirement for the development of an occlusive thrombus.

In recent years, considerable research has been directed toward elucidating this problem of the chicken and the egg: what part does coronary occlusive thrombi play in myocardial infarction? is it a primary or secondary mechanism? does occlusive coronary thrombus precede or follow the occurrence of myocardial necrotic areas? As in many other fields of atherosclerosis research, too many investigators believe that in order for one point of view to be correct, the others must be incorrect. It should be stressed first of all that the apparently illogical development of occlusive thrombosis after infarction has not yet been adequately explained; likewise, it has not been clearly demonstrated that an occlusive coronary thrombus has no primary role in the pathogenesis of myocardial infarction.

The occlusive coronary thrombi and myocardial infarctions are clearly related, since the thrombus is frequently located in the artery supplying the infarcted area. On the other hand, the nature of this relation seems to be very complex, the occlusive coronary thrombi acting either as a cause of the myocardial infarctions or appearing as a consequence of the onset of large areas of myocardial necrosis. The classical concept is continually supported by angiographic studies performed after the clinical onset of a heart attack; in certain of these studies, the incidence of occlusive thrombi was demonstrated to decrease with time, for instance, 87% within 4 hr of the onset of symptoms and only 65% at 12 to 24 hr after the onset.[129] These results suggest that an occlusive thrombus able to give rise to a myocardial infarction may fragment or resolve within a few days. This capacity of occlusive coronary thrombi to resolve rapidly is also supported by the facility with which therapeutic interventions may lead to thrombus dissolution.[130]

The complexity of the pathogenetic mechanisms involved in human myocardial infarction is reflected by the fact that occlusive coronary thrombi are frequently associated with transmural myocardial infarction and cardiogenic shock, while subendocardial myocardial in-

farction without shock is rarely associated with coronary thrombosis. In many patients who died of sudden cardiac death coronary thrombosis may be present in the absence of myocardial infarction; on the other hand, myocardial infarction without coronary occlusive thrombosis is not a very rare occurrence. Particularly in young subjects, myocardial infarction may result from mechanisms not related to the presence of obstructive atherosclerotic lesions, such as coronary vasospasm, coronary emboli, platelet microthrombi, small vessel disease, idiopathic myocarditis, toxic cardiomyopathy, anatomic abnormalities of the coronary arteries, myocardial steal, abnormal oxygen-hemoglobin dissociation, abnormal lysosomal enzyme activity, microcirculation disturbances, neurocirculatory asthenia, increase in catecholamines, altered cell membrane permeability, and local changes in potassium, magnesium, sodium, and particularly in calcium.

Experimental studies revealed that an occlusive thrombus may be associated with a full thickness infarct, whereas mural, nonocclusive thrombi may give rise to focal ischemic myocardial lesions of a variety of types similar to those found in the hearts of individuals who died suddenly of a presumed coronary event.[106] These include myofibrillar degeneration, focal necrosis of myocardial fibers, interstitial round cell infiltration, and small infarcts. The attention of investigators was mainly centered on coronary vasospasm able to complete a stenotic lesion or a thrombotic occlusion. Here again, the same problem of the chicken and the egg: what part does spasm play in these events? is it a primary or secondary mechanism? might spasm lead to plaque disruption or once disruption of the surface of the plaque has occurred, the rapid aggregation of platelets and of TXA_2 release promote coronary vasospasm? Both sequences could render the myocardium increasingly sensitive to ischemia, and if collateral vessels are not available, severe myocardial damage and even irreversible necrotic lesions might occur.

The close relationship between plaque rupture and coronary occlusive thrombosis is more difficult to explain on the basis of postinfarction thrombosis. On the other hand, if we accept the view that an occlusive coronary thrombus is already compensated for by collaterals, neither the so-called obstructive thrombosis, nor the rupture of the necrotic center of the plaque, intraplaque hemorrhage, or another important change leading to total occlusion of the lumen of a major coronary artery will have any clinical and pathological significance.[131] This also would mean that certain attempts to produce lysis of an occlusive thrombus in patients with well-developed collateral vessels are questionable. Such patients could be compared with those which present a thrombotic occlusion of a major coronary artery in which severe stenotic plaques have been successfully bypassed surgically by an aortocoronary vein graft. This existence of occlusive thromboses without functional significance and clinical meanings, in spite of their development in the coronary arterial tree, is a new concept in cardiology;[131] it may lead to the opinion that the efforts to preserve and develop large collateral vessels, able to adequately bypass an area of thrombotic occlusion, could be in many cases more important than the effort to produce thrombolysis.

In the available literature, *the reported frequency of occlusive thrombosis among fatal myocardial infarction ranges from 0 to 96%*. This unexpected discrepancy might be attributed to a technical fault, differences in the selection of patients, a different definition of myocardial infarction, a possible spontaneous lysis of the occluding thrombi, and to many other general and local factors. If we refer, for instance, only to the definition of myocardial infarction, it might be of interest to mention that if we accept that the area of necrosis must be at least 25 mm in length, then the frequency of occlusive coronary thrombosis may range from 80 to 90% and this might help to assume that thrombosis comes first. If the definition of myocardial infarction includes small areas of subendothelial necrosis, the frequency of coronary occlusive thrombosis may range from 0 to 20% and the conclusion could be that myocardial infarction comes first. The available literature includes many papers which report myocardial infarctions with severe atherosclerotic involvement of the coronary arteries and

also with normal or patent coronary arteries. This could lead to the opinion that there are at least two main types of myocardial infarctions, a classical type originating in coronary thrombotic occlusion and an idiopathic type, with or without severe atherosclerotic involvement of the coronary arteries. Our own experience, as well as the conclusions of many other experienced pathologists, would indicate that the frequency of myocardial infarctions in cases with apparently normal coronary arteries or even with so-called mild atherosclerotic involvement is unimportant, the magnitude of this problem being overemphasized for reasons which are not clear. About 1 decade ago, this magnitude was convincingly estimated at less than 1% of cases.[132] If one uses estimates of frequency in young patients, this proportion may augment to 10%, but if one uses patients of all ages, this proportion falls dramatically to only 2%.[133] According to certain observations, 1 to 7% of cases of myocardial infarctions examined either angiographically during life or at autopsy will have coronary arteries without demonstrable severe stenotic or occlusive atherosclerotic plaques; the converse of these estimations is that 93 to 99% of cases will have severe stenotic or occlusive atherosclerotic plaques.[134] The point to be made is that the association of myocardial infarction with angiographically normal coronary arteries could be considered an unproved combination.[132] If an association exists, it must be centered on acute coronary thrombosis with subsequent clot lysis, retraction, or recanalization, each of which may appear as angiographically normal. An occluding thrombus which subsequently lyses or is recanalized can be considered a major cause of myocardial infarction in patients who are subsequently shown by angiography to have normal coronary arteries. It is reasoned that the coronary arteries were not normal at the time of the acute stage of myocardial infarction, but that the occluding lesion resolved between the time of the onset of clinical manifestations and the time of coronary angiography. The rapid regression to near normal of the ECG changes several days after the infarction might also support lysis of the clot. In this respect it might be of interest to recall the case of a patient with an acute anterior wall infarction, as documented by clinical, ECG, and enzymatic evidence. The disappearance of the infarction pattern on the ECG was recorded 5 days after the onset of the clinical manifestations. The next day, the patient died suddenly and on post-mortem examination, the coronary arteries showed only slight atherosclerotic involvement, despite the presence of a transmural anterior infarction with a mural thrombus.[135]

In essence, the demonstration of a coronary thrombotic occlusion associated with a myocardial infarction does not prove that the occlusion is always the first event and each case must be studied as a separate entity. In each patient investigated, the problem which comes first, coronary thrombosis or myocardial infarction, could represent a subject of possible dispute. It might be magnified by nosological and semantic differences, methodological heterogeneity and/or inadequacy, and the bias inherent in the selection of subsets of the patients for the study. Occlusive coronary thrombosis seems to play a primary role in the pathogenesis of large transmural myocardial infarctions; in these patients, the absence of an occlusive lesion long after a thromboembolic episode does not mean that no occlusion was present at the time of myocardial infarction. On the other hand, the infrequency of coronary occlusive thrombi in patients who died of acute cardiovascular collapse, without myocardial necrosis, or in those in whom necrosis is limited to the subendocardium cannot be overlooked. Likewise, the infrequency of occlusive coronary thrombi in patients who died without cardiogenic shock or congestive heart failure and their occurrence at, or proximal to, sites already severely narrowed by old atherosclerotic plaques is worth mentioning. A number of fundamental questions remain to be answered in this field of research and it may be of interest to recall two significant cases in which surgical ligature of the bleeding left anterior descending artery due to chest injury in young, healthy men, was not followed by the occurrence of myocardial infarctions.[136,137]

C. Collateral Vessel Changes in Myocardial Infarction

Necropsy studies have shown that the infarction size is usually smaller than the perfusion

area of the obstructed coronary artery and many cases of total coronary occlusion with no myocardial damage have been reported. Both types of observations are related to the existence, in the human heart in any cardiac region, of collateral vessels with a diameter ranging from 20 to 350 μm. Many data on the anatomy, physiology, pathophysiology, and angiographic aspects of these collateral vessels are presented in Chapter 2.

Coronary collaterals are thought to be genetically determined in each individual, but they do not appear to be functional in the absence of a severe degree of luminal obstruction, usually more than 75%. When this obstruction develops, a low pressure region appears distal to the obstructed area and the gradient of pressure between the vessel segments proximal and distal to the stenotic plaque favors collateral enlargement and their role as bypass vessels.

More than 3 decades ago, the significance of collateral circulation in relation to the onset of both subendocardial and transmural myocardial infarctions was emphasized.[138] This study revealed that with the onset of infarction, collateral vessels modify their caliber, course, and number and in certain investigations this increase in number was considerable — more than 14 times.[139] A correlation was established between the number of collateral vessels and the number of major coronary arteries involved by stenotic atherosclerotic plaques, augmentation in the number of intracoronary anastomoses (which connect segments of a similar vessel) being greater than that of intercoronary anastomoses (which connect different vessels of the coronary arterial tree).

The time required for collaterals to enlarge in response to an acute occlusive lesion developed in a major coronary artery or in a main branch vessel in man can only be suggested. In dogs, this enlargement could be followed step by step and required 1 to 4 days. Likewise, in experimental models it was possible to demonstrate that *when coronary stenosis was produced gradually and an adequate collateral network developed, both myocardial necrosis and lethal arrhythmias rarely occurred.* Conversely, when coronary stenosis occurred abruptly, collateral flow to replace the flow furnished by the obstructed major coronary artery was inadequate, and both myocardial necrosis and lethal arrhythmias were present. In certain studies in dogs, the coronary blood flow appeared greater in progressive gradual stenosis than in abrupt total occlusion by only 35%.[140] This difference is statistically significant and the results of this experiment demonstrate than even a relatively small increment in collateral flow seems to be functionally important.

The ability of collateral vessels to protect jeopardized myocardium is obvious for all experienced pathologists, but this ability varies from case to case, since each heart has its particular anatomical coronary branching pattern and its particular network of collateral vessels. This could be more clearly demonstrated in laboratory animals, since some species lack a collateral vessel system, and in such species a linear relationship can be established between the degree of luminal narrowing of a major coronary artery and the amount of myocardial mass which becomes necrotic as a result of blood supply interruption. On the other hand, in species with a well-developed collateral network this linear relationship could not be demonstrated, the severity of luminal narrowing losing its ischemic significance. The great majority of human beings possess a well-developed collateral network and therefore the real significance of the degree of luminal narrowing is difficult to establish. *The anatomic boundaries of the region of the myocardium supplied by the occluded vessel is equivalent to the myocardium at risk of infarction only in the absence of an adequate collateral circulation.* Theoretically, all regions which continue to receive blood from collateral vessels may be considered salvageable myocardium. Simply stated, the amount of collateral flow is the main factor which determines the extent of myocardial infarction in all subjects with an adequate development of collateral vessels. There are coronary heart disease patients with good collaterals on the angiograms obtained during acute myocardial infarction; these patients do not present cardiogenic shock and usually are not in real danger. On the other hand, the patients with poor collaterals show cardiogenic shock and increased mortality.

These and many other observations strongly suggest that the quality of collateral circulation is an important determinant of the clinical evolution of an acute myocardial infarction.

Neither in vivo angiography nor routine post-mortem examination are able to account for the variation in collateral flow from case to case and from vessel to vessel. We cannot estimate the number and size of collaterals in human hearts without the use of special and time-consuming methods, and even if the angiographist is able to detect some large collateral vessels, he cannot demonstrate their real number and particularly their intramyocardial networks. These intramyocardial networks of collateral vessels seem to play the main role in persistence of a subendocardial area of necrosis as such, or in its progression toward a transmural infarction. Theoretically, whenever an inadequate collateral circulation exists in the subepicardial region, a transmural extent of necrosis is to be expected. Experimental data also suggest that the transmural extension of a small subendocardial area of necrosis seems to be predetermined by the availability of collateral flow. In our material, the extent of the wavefront of necrosis from subendocardial to subepicardial region seemed also to be related to the availability of collateral flow and especially the occlusion of collateral branch mouths by thrombi. This phenomenon was mainly observed in the intermediate segment of the right coronary artery between the points of origin of the right marginal and posterior descending branches. Both proximal and distal growth of the occluding thrombus might obstruct the branch mouths of some important collaterals and thus might hamper the circulation of the ischemic zone distal to the occlusion. This may be followed by an extension of the necrotic area. Moreover, a vicious circle seemed to develop, since the extension of the wavefront of necrosis could also involve the terminal segment of some collateral vessels and create a zone inaccessible to blood flow. A decrease in the gradient of pressure necessary for an adequate flow through collateral vessels and even the possible occurrence of steal phenomena appear. Since the blood flow distribution is chiefly dependent on pressure gradients, it is likely that the blood flow, when prevented from running in an area with an increased peripheral resistance, will escape in the adjacent vessels with lower resistance. *This flow redistribution could represent an important mechanism in the passage from a subendocardial to a transmural infarction or from localized to circumferential infarction.* In such hearts with coronary steal, the myocardium at risk cannot be adequately determined and it is possible to detect the so-called paradoxical infarcts or infarct at a distance.

The frequent thrombotic occlusion of a stenotic atherosclerotic plaque bypassed surgically by patent aortocoronary vein graft would indicate that thrombosis may develop in the presence of an adequate vascular supply of the myocardial area at risk. This and many other similar observations suggest that there are secondary progressive thrombotic occlusions of the coronary arteries with or without a particular functional and clinical meaning. These thrombi develop in the presence of the myocardial area at risk, partly or totally compensated for by a patent aortocoronary vein graft or by an adequate number of physiologically developed collateral vessels. Such thrombi which extend proximal and distal to the critical lesions lack an occlusive function as long as an adequate compensatory circulation exists, but acquire this occlusive function if the aortocoronary bypass vein graft is significantly narrowed, or if the branch mouths of important collateral vessels are obliterated. An angiographer who records the absence of collaterals may in fact visualize a case in which large collaterals exist, but their branch mouths were obliterated by the extension of mural thrombi. This misinterpretation may be a source of other mistaken conclusions. It is perhaps also of interest to repeat once again that even when the branch mouths of collateral vessels are free, coronary angiography is usually unable to detect vessels with a diameter less than 100 to 200 μm, nor can it detect intramural collateral vessels. Therefore, this method has a limited capacity to demonstrate the real presence of collateral networks and their changes related to the development and evolution of myocardial infarctions. These method-limitations in the visualization of collateral vessels by coronary arteriography have obliged investigators to use

other additional techniques, such as those that reveal changes in left ventricular motion and function, as well as changes in electrophysiology and radionuclide myocardial imaging. *The ease with which certain investigators record the angiographic absence of collaterals as a true anatomical absence is unfortunate.* When this absence of collaterals is mentioned, then the degree of luminal narrowing acquires a magic value, which may be real only in subjects with a genetic absence of collaterals. In addition, all therapeutic interventions which try to reduce myocardial hypoxia might retard collateral development if we accept the view that transient myocardial hypoxia is an important stimulus for both collateral development and enlargement. A chronic reduction in myocardial metabolic requirement might also retard collateral development and enlargement. Finally, indirect evidence as to the effectiveness of myocardial revascularization in resolving the existence of large areas of myocardial ischemia is the angiographic regression of collaterals. This would indicate that when the stimulus produced by myocardial ischemia on collateral vessel development and enlargement is removed, the result would be angiographic disappearance of collateral vessels. It might be also of interest to note that the protective role exerted by collateral vessels could be more clearly demonstrated in cases with anterior infarction and complete obstruction of the left anterior descending artery.[141]

In essence, three basic mechanisms seem to influence the onset or limit the size of the myocardial infractions:[131,142,143] (1) the rate of development of large collateral vessels from preexisting collateral networks of the coronary arterial tree, (2) the number and caliber of collateral vessels available at time of coronary occlusion, and (3) the amount of myocardium supplied by these collaterals and by the occluded coronary artery. The rate of collateral development, stimulated by gradual occlusion of the coronary artery lumen, appears in many works as the critical factor in limiting infarct size. This development requires the persistence of an adequate gradient of pressure between the proximal segment of the major coronary artery which gives rise to collaterals and the myocardial ischemic area. This adequate gradient of pressure is an important and necessary factor providing blood flow to the impaired vascular bed during the long period of occlusion; it is also an important and necessary factor for the transport of drugs and other chemical means of protecting the ischemic myocardium, since such chemicals are actually transported to the ischemic or necrotic areas via the collateral circulation. Therefore, enhancement of collateral flow is a desirable goal, but unfortunately in certain experimental models, chronic β-adrenergic suppression by propranolol was associated with an inhibition of collateral vessel development.

A topic which cannot be neglected is the size of the perfusion territory of the occluded coronary artery. It is tempting to presume that in the presence of a short left circumflex artery (which supplies a small volume of the lateral wall of the left ventricle), if the vessel is occluded the collateral circulation will compensate for an important part of the perfusion territory of this small, short left circumflex artery. In the presence of an occlusive plaque developed in a large and long left circumflex artery which supplied the lateral and posterior walls of the left ventricle and a part of the interventricular septum, the compensation offered by the collateral circulation may be inadequate. *This example suggests that the myocardial infarction size is largely influenced by the relationship between the amount of collateral flow available and the size of the vascular bed at risk.* An abundant collateral flow supplying a small vascular bed might be associated with the absence of infarction in spite of the presence of occlusive atherosclerotic plaques; the same bed site, but with a significantly reduced collateral flow may develop ischemic necrosis. It might be assumed that the most important factor related to the magnitude of an area of ischemic necrosis is not the size of the perfusion territory or the abundance of collateral flow taken as separate entities, but the ratio of the two parameters perfusion territory resistance vs. collateral vessel resistance. This ratio increases with the severity of the stenotic lesion and decreases with enlargement of the collaterals.[143] This enlargement of preexisting collaterals may restore perfusion of the myo-

cardium toward normal and this could explain the association on post-mortem examination, of an occluded major coronary artery and an apparently unaltered myocardium. Likewise, this enlargement of preexisting collaterals may create local conditions for the penetration of drugs, an adequate coronary reserve, and may diminish the likelihood of myocardial infarction in the event of subsequent total occlusion of a preexisting stenotic atherosclerotic plaque.

D. Myocardial Ischemic Events Induced by Coronary Atherosclerosis

1. Reversible and Irreversible Cell Damage

During the past decades, many studies have analyzed in detail the changes in cellular properties and implicitly in heart metabolism associated with the passage from the normal state to reversible and irreversible damage occurring as myocardial ischemic necrosis. Both experimental and human studies show that myocardial ischemia is a very complex phenomenon, and includes a succession of intricated events. This complexity is also reflected in the number of theories and viewpoints which try to explain the passage from reversible to irreversible changes and in the number of propositions to prevent ischemic myocardial cell death.

The alterations (functional, metabolic, structural) associated with ischemia have been mainly evaluated in experimental models, the most largely used being the production of regional myocardial ischemia following coronary artery mechanical occlusion in vivo. Other experimental models are based on total ischemia in excised hearts, thin myocardial tissue slices, isolated preparations of contractile cells, or isolated perfused hearts or septa. Likewise, various types of substrate deprivation and/or metabolic inhibitors have been largely used. The results show that the heart is strongly dependent on aerobic metabolism for energy production, and when myocardial supply is reduced to the point where energy requirement and particularly oxygen availability for oxidative phosphorylation are inadequate, the myocardium becomes ischemic. The principal initial events in myocardial ischemia seem to be

1. Insufficient oxygen supply in the pericellular space of myocardial cells
2. Deprivation of myocardial cells of the supply of substrates necessary to produce energy
3. Inability of the coronary blood flow to remove products of myocardial cell metabolism

About 3 decades ago, the following sequence of events was proposed to explain the influence of myocardial ischemia on cardiac functions in subjects with stenotic or occlusive atherosclerotic plaques:[144]

- Reduced blood supply
- Accelerated glycolysis
- Abnormal lactic acid production
- Intracellular acidosis
- Displacement of calcium ion bound to troponin during systole
- Failure of activation of the actin-myosin system
- Impaired contractility
- Systolic bulging of the ischemic ventricle

Subsequent studies have revealed that ischemia sets into motion a complex series of events, involving every organelle and subcellular system, and having as their starting point deprivation of oxygen and ATP production. This involves changes in ion transport by plasma membrane, alteration of protein and nucleic acid synthesis, cessation of fatty acid oxydation, and autolytic events related to several types of hydrolases present in various cell compartments.[145]

As a first step, analytical investigations have been mainly centered on mitochondria, since mitochondrial function appears to be rapidly arrested following ischemia, cellular oxygen levels rapidly approach very low values, and ATP synthesis, electron transport, and other functions of the mitochondria are damaged. Of particular importance seems to be changes in mitochondrial membrane permeability, an increase of this permeability promoting proton leak, and reducing oxidative phosphorylation. Transmembrane ionic imbalance, leakage of macromolecules, and loss of mitochondrial components were correlated with a loss of cell viability.

There are many other mechanisms suggested to be involved in the onset of myocardial ischemia and in the progression of lesions from reversible to irreversible stages. While not denying their possible importance, we should adopt a cautious attitude, since the results are mainly based on experimental models, and in man this is an area still in its infancy. Space limitations do not permit consideration of this fascinating subject; consequently, only some salient data are presented on the changes in calcium ion and ATP levels, on the injury produced by accumulations of free radicals, and by release of lysosomal enzymes.

It might be of interest to recall that the normal myocardium relies principally on free fatty acids for its nutrition both at rest and during exercise. When severe ischemia develops and the available oxygen is insufficient for the oxidation of free fatty acids, an energy crisis could result. Moreover, some free fatty acids may become toxic to the ischemic myocardium and may favor lethal arrhythmias of the ventricular fibrillation type. In addition, a local abnormal level of free fatty acid could induce an extension of the infarction area. Likewise, high levels of lactate and hydrogen ions interfere with oxidation of NADH and are potentially detrimental to myocardial function and metabolism. In certain studies, emphasis is placed on this accumulation of metabolites in producing the biochemical defects in glycolytic regulation found in ischemic hearts.[146]

The attention of many investigators has been focused on adenosine triphosphate (ATP) since it is essential for myocardial contraction, providing not only the energy for this contraction, but also the maintenance of ion gradients and adequate synthesis of macromolecules. Therefore, ATP depletion may be the primary trigger for early contractile failure. Sites where decreased ATP levels might influence mechanical activity include myosin-ATPase, the sarcoplasmic reticulum Ca^{2+} pump, and sarcolemmal Na^{+}-K^{+} ATPase. This could explain why reduction in myocardial blood flow sufficient to compromise myocardial oxygen requirements seriously impairs energy production by the heart. Maximum activity in such conditions represents only a small percentage of energy requirements. Although the glycolytic pathway in cardiac muscle is very active, it is unable to keep pace with the energy demands of the contracting heart. Endogenous lipids are used in preference to glucose even though the exogenous glucose concentration is higher than that necessary to achieve maximum rates of glucose utilization. When free fatty acids are present, their oxidation predominates and accounts for most myocardial ATP production. At the onset of myocardial ischemia, glucose utilization may also account for the ATP produced; in addition, hydrolysis of stored ATP and creatine phosphate, together with enhanced glycolysis primarily due to augmented glycogenolysis, may compensate for the decreased supply of oxygen for a short period of time. When creatine phosphate and glycogen stores are depleted, the glycolytic rates of ischemic hearts decrease to that of aerobic hearts. This loss of tissue high energy phosphate stores with the onset of ischemia reflects the inability of the contracting heart to produce adequate amounts of ATP. On the other hand, the rate of ATP utilization is reduced in the ischemic cell and this could explain why ATP is still present in many of these cells and why they appear able to maintain certain functions for a significant period of time, as well as the reversible character of the lesion. The linear relationship between loss of ATP and loss of cell viability is not clearly demonstrated, but is largely accepted. Many investigators maintain that irreversibility occurs at some time after ATP falls below a critical

level, but cell death is associated with more complex changes: depletion of both cellular ATP and the adenine nucleotide pools, acidosis, slowing and eventual cessation of anaerobic glycolysis, and a variety of ultrastructural changes, including disruption of the sarcolemma.[147]

A possible association between ischemic episodes and the massive increase in calcium ions was also suggested.[148] According to certain views, maintenance of intracellular homeostasis with respect to calcium ions during prolonged episodes of ischemia and upon reperfusion appears critical to the survival of the myocardium.[149] One of the most widely accepted hypothesis focuses on membrane damage as the primary event. As a consequence, excess calcium ions enter the myocardial cell and critical intracellular components such as enzymes and cofactors leak into the extracellular space, resulting in an irreversible injury. The early net gain in calcium ions probably occurs because of an ATP-depletion-induced failure of the Na^+-K^+ ATPase to extrude sodium ions against the prevailing concentration gradient. An additional net gain in calcium ions appears to result from the entry through sarcolemmal defects and/or slow channels. In this respect, note the demonstration that nitroglycerin stimulates Ca^{2+} extrusion from vascular cells, which is operated by the sarcolemmal Ca^{2+} pump ATPase.[150] The consequences of a raised calcium ion level are ATP wastage, irreversible loss of mitochondrial function, enhanced phospholipase, and proteinase activation. The following sequence of events has been proposed to explain an enhanced entry of calcium ions:[149] ATP-depletion induces failure of Na^+-K^+ ATPase, the ischemic myocardium fails to extrude sodium ions against the prevailing concentration gradient, the tissue sodium ion level rises leading to an activation of the Na^+:Ca^{2+} exchange mechanism, sodium ions will leave the tissue in exchange for calcium ions. This gain in calcium ions seems to appear early during the ischemic event and the heart must be protected against the consequences of a raised calcium ion levels.

With the onset of myocardial ischemia, there is not only activation of membrane bound phospholipases, but free radicals are also produced at an enhanced rate and long chain fatty acids accumulate. A number of experimental models have shown abnormally high concentrations of cytotoxic oxygen-derived free radicals and decreased concentrations of protecting antioxidizing enzymes during myocardial ischemia. These free radicals react with the unsaturated lipid of the cell membrane to produce very toxic endoperoxides.[151,152] The ability of scavengers of oxygen free radicals (superoxide dismutase, catalase, mannitol, etc.) to improve mechanical, mitochondrial, and sarcoplasmic reticulum function in animal models of myocardial ischemia, and ischemic reperfusion injury suggests that oxygen free radicals are partly responsible for tissue alterations in these models.[153] These tissue alterations are very complex and among such complex changes it might be of interest to mention loss of mitochondrial oxidative phosphorylation and calcium sequestration by the sarcoplasmic reticulum. Note also membrane injury by peroxidation of polyunsaturated fatty acids within the phospholipid structure of the membrane. Free radicals also bind to membrane enzyme receptors, thereby modifying the activity of membrane components and causing disturbances of transport phenomena![154] Free radicals may inhibit PGI_2 synthetase, may disturb PGI_2-TXA balance, and may favor platelet aggregation in small intramyocardial vessels which aggravates myocardial ischemia. In certain experimental models, the damage produced by free radicals appeared of particular importance in the reperfused ischemic myocardium, when generation of such free radicals in the high oxygen environment of the reactive hyperemia of reperfusion could kill potentially viable cells reversibly damaged by ischemia.

Over the past decade, a certain amount of interest has focused around the hypothetical involvement of lysosomes in myocardial ischemic cell injury.[155-157] An editorial on this topic has a very suggestive title: "Lysosomal alterations in ischemic myocardium: result or cause of myocellular damage?"[158] It is largely agreed that myocardial ischemia and its consequent intracellular acidosis may disrupt lysosomes and allow acid hydrolases to gain access to the cytosol and to produce cell injury and cell death. The leakage of hydrolytic enzymes begins

early in ischemia and continues during the whole ischemic period, degrading macromolecules to biologically active peptides which may be significant in causing inflammation and the extension of necrotic areas. As ischemia progresses, continuity of the lysosomal membrane is usually lost and diffusion of enzymes is not difficult to detect. Some investigators think that this activation of the enzymes of cardiac cell lysosomes is the "point of no return" in myocardial ischemia. This hypothesis has been questioned since some studies have shown that myocardial cells contain few lysosomes and that a heterogeneity exists in the lysosome response to ischemia. It is thus possible to consider lysosomal activation as only a secondary change in the cascade of events leading to cell death. On the other hand, experimental evidence shows that the ability of steroidal anti-inflammatory agents to retard myocardial injury after coronary occlusion may be related to their lysosomal stabilizing effect; also nonsteroidal anti-inflammatory agents seem to have a beneficial effect on myocardial ischemia. In other words, is it necessary to stabilize lysosomes by therapeutic interventions in order to prevent the death of cells during myocardial ischemia? The topographic relationship between hydrolase redistribution and irreversible cell changes has been shown in few experimental models and the coexistence of the two events does not prove causality. The possibility exists that lysosomal changes are merely coincident with, not a cause of, irrevesible changes. These changes may appear before lysosomal enzyme redistribution. On the other hand, ischemia produces lowered ATP, lowered pH, accumulation of membrane active fatty acids, and a reduction in the integrity of lysosomal membranes. This is followed by a dispersion of lysosomal enzymes and by abnormal breakdown of cellular constituents. If severe enough, this abnormal hydrolysis of cellular constituents could contribute to the development of cell damage and, ultimately, cell necrosis.[155,158]

Summing up, decreased coronary flow induced by the presence of stenotic or occlusive atherosclerotic plaques of the coronary arteries is involved in both altered oxygen delivery and removal of metabolic products. Of particular importance is ATP conversion to ADP and AMP, hydrolysis of AMP by 5′-nucleotidase associated with loss of total adenine nucleotide, and the negative influence exerted by low ATP levels on many energy-dependent reactions, including ion pumping and calcium ion sequestration. Irreversible changes seem to result when ATP levels are too low to support critical cellular functions and particularly myocardial contraction. The rapid fall in contractility could be related to an interference with the calcium cycle produced by the decrease in intracellular pH. This fall in intracellular pH and the accumulation of compounds with detergent activity may enhance membrane damage. In a final step, the lysosomal system is activated and this activation is associated with the disruption of intracellular membranes.[146]

2. Particular Features of Myocardial Necrosis

A coronarogenic myocardial necrosis implies primary mechanical impairment at some point of the coronary arterial tree which interferes with the myocardial blood supply. This mechanical impairment is usually produced by the development of a stenotic atherosclerotic plaque associated with an occlusive thrombus and with an inadequate development of collateral vessels. The myocardial area distal to this lesion is rendered ischemic and cell death may follow, myocardial necrosis being identified 4 to 6 hr after the onset of coronary occlusion. In addition to stenotic atherosclerotic plaques, a coronarogenic myocardial necrosis can also be produced by coronary vasospasm, intramyocardial platelet aggregates, and other mechanisms able to imair coronary blood flow. Many techniques have been proposed to outline the early stages of ischemic myocardial injury, with particular emphasis on the characteristics defining reversible and irreversible injury.

There are also noncoronarogenic myocardial necroses produced by a direct injury to the contractile cell by catecholamines or during infections and allergic states, toxic conditions, severe metabolic and hormonal disorders, anoxia, anemia, shock, etc.

In both coronarogenic and noncoronarogenic myocardial necroses the prevalent lesion is myocytolysis, macro- and microfocal, able to give rise to various types of myocardial clinical manifestations.

Particular emphasis is placed in this book on coronarogenic myocardial necrosis produced by severe atherosclerotic involvement and occurring as an end stage in the natural history of coronary atherosclerosis. According to a largely agreed concept, in such types of necroses, the blood supply to the myocardium is limited or totally interrupted by stenotic or occlusive atherosclerotic plaques associated with the absence of adequate collateral vessels, myocardial necrosis being the result of a progressive and severe ischemia.

Baroldi suggests that the myocardial cell may die in a state of irreversible relaxation (atonic death), irreversible contraction (tetanic death), and finally, with progressive loss of its function (failing death).[131,159]

Atonic death occurs microscopically as a coagulation necrosis and represents the pathognomonic post-mortem finding in patients dying from typical acute myocardial infarction.[159] In the majority of cases the lesion is monofocal, its size ranging from less than 10% to more than 50% of the left ventricular mass. In atonic death, the muscle fibers show thinning, the nucleus elongated, pyknotic, and progressively fading, the elongated myofibril sarcomeres appearing in normal registered order. A particular feature of this type of myocardial necrosis is a massive polymorphonuclear infiltration. The contour line where the polymorphonuclear infiltration stops being the morphological sign of blockage of flow around the noncontracting area. These polymorphonuclear leukocytes undergo relative rapid lysis and disappear within the first week. The cells which die in an atonic state show severe involvement of the mitochondria and nucleus, but without evident damage of the myofibrillar system. On the other hand, this type of necrosis is associated with passive overdistension due to intraventricular pressure and systolic bulging. Such negative hemodynamic effects can be compensated by a hyperfunction of the surrounding viable contractile cells, especially those located at the borderline of the noncontracting region. As concerns the repair process, it seems to be carried out mainly by macrophage activity rather than by formation of granulation tissue. The vessels included in the necrotic area are also submitted to destructive changes with secondary hemorrhage and thrombus formation. This atonic death or coagulation necrosis can be revealed in all areas of myocardial infarction, particularly in the central zone, but appears in only 17% of cases of sudden cardiac death.[131]

The second type of myocardial necrosis, designated as tetanic death, coagulative myocytolysis, or Zenker's necrosis, is characterized by an irreversible contraction that prevails in the external layer of myocardial infarctions, but is also present in the apparently normal myocardium. It seems to start with hypercontraction of muscle fiber or a segment of it and is associated with stretching and breakdown of the myofibrils. Abnormal irregular transverse bands are present, variable in size and shape, and are considered to represent a coagulation of hypercontracted sarcomeres.[142] This is associated with an increase in their affinity for acidophilic stains, followed by progressive disappearance of the contractile apparatus. With the progression of this lysis of hypercontracted sarcomeres, an empty sarcolemmal tube appears. In areas with coagulative myocytolysis, no early infiltrates were seen and no polymorphonuclear leukocytes were present. Only mononuclear cells were detected at times (mainly lymphocytes); also, the vessels within the lesions preserved their microarchitecture. It would be of interest to recall that coagulative myocytolysis was revealed in cases of pheochromocytoma and in experimental catecholamine-induced necrosis. This would indicate a possible primary role of catecholamines in the pathogenesis of this type of myocardial necrosis.[131,142]A sympathetic overstimulation may determine an irreversible contractile state with myofibrillar damage by a massive intracellular penetration of calcium ions with depletion of high energy phosphate stores.

A third type of myocardial necrosis is called failing death or coliquative myocytolysis.[142]

The prevailing change is dissolution of the myofibrils secondary to an edema-like intracellular fluid appearance followed by progressive vacuolization, and leading to empty, dilated, sarcolemmal tubes. This type of necrosis was mainly seen in the subendothelial region, being present in less than $^1/_2$ of myocardial infarcts.

Studies centered on the onset and fate of these three types of myocardial necroses revealed that the extension or reduction of an infarct size is due rather to the variation of coagulative myocytolysis (tetanic death) than to a change in volume of the primary areas of coagulation necrosis (atonic death). Coagulative myocytolysis also seems to be the form of myocardial necrosis most deeply involved in the onset of ventricular fibrillation and sudden cardiac death.

Infarct necrosis or the so-called coagulation necrosis appears as the form of myocardial damage most likely to be caused by reduction of the nutrient flow produced by stenotic or occlusive atherosclerotic plaques. As concerns the pathogenesis of Zenker's necrosis or coagulative myocytolysis, it is mainly the result of metabolic abnormalities produced by excessive depletion and release of catecholamines. This would mean that these forms of myocardial necroses must be considered as distinct entities and not as stages in the evolution of the same type of injury. Consequently, a correct determination of the size of a myocardial infarction produced by occlusive coronary atherosclerotic plaques would require measurement of the tridimensional extension of the areas involved by coagulation necrosis.[159]

In spite of the apparent complexity of the various forms of myocardial necrosis, once the infarct is established, the chronologic staging of the lesion can be followed with surprising accuracy.

In human myocardial infarction, an inflammatory response is initiated in the immediately adjacent viable tissue during the first week. During the second week, monocytic infiltration predominates, macrophages moving into the dead tissue which is progressively removed by a combination of proteolytic enzyme liquefaction and macrophage activity. It has been postulated that macrophages also stimulate the proliferation of fibroblasts and capillary beds. Granulation tissue with an abundant supply of newly formed blood vessels extends into the necrotic area from about the end of the first week, and in favorable conditions ultimately replaces the necrotic muscle completely. Collagen fibers also develop in the advancing granulation tissue from about the second week onward, and after week 4, there is a progressive fibrosis with ulterior shrinkage and devascularization, until the lesion becomes a mature myocardial scar. Residual activity and even necrotic areas may persist in very large infarcts dispersed throughout a stellate, opaque, and contracted tissue. This healed infarct is menaced with rupture, but once the scar has developed, the orientation of the collagen fibers and their macromolecular stabiltiy could explain why rupture does not frequently occur. On the other hand, the scar is subjected to outward pressure and aneurysm formation, as well as thrombus development. In essence, acute inflammatory changes predominate during week 1, a chronic inflammatory peak can be detected during week 2, and prevalent connective tissue proliferation during week 3.

It might be of interest to note that:

- At 48 hr 100% of the myocardial infarcts are surrounded by a distinct zone of polymorphonuclear leukocytes
- Lymphocytes do not appear at the edge of the infarction area until the second day and from the sixth day on are present in 100% of infarcts
- Mononuclear macrophages appear by the third day and plasmocytes by the fourth day; from the seventh day on they are present in 100% of infarcts
- Hemosiderin-laden macrophages do not appear until the seventh day and may persist 1 to 3 months
- Eosinophils are present in only one out of every three to four myocardial infarctions

- Cellular infiltration is always less on the subendothelial margin of the lesion than on the other margins
- By the second day all areas of myocardial infarction may exhibit irreversible changes, particularly loss of recognizable cellular organelles
- Many apparently necrotic cells retain cross-striations

3. Development of a Border Zone

The term "border zone" has become a confusing and controversial one, partly because it has been used to denote different changes by different investigators. It usually delineates a myocardium that is sufficiently ischemic as to become irreversibly injured in the absence of an adequate therapy, but still viable and thus potentially salvageable when an adequate therapy is initiated. This term refers mainly to the margins of evolving myocardial infarcts, showing intermediate severity of ischemia and implicit cellular injury, this severity being strongly related to the adequacy of collateral circulation.

The term border zone is also used to refer to an area of the myocardium consisting of mixtures of interdigitating normal and necrotic tissue, located at the periphery of a large necrotic area, or to a region of viable myocardial cells located at a certain distance from the necrotic area but considered at risk of death if deterioration of the oxygen supply-metabolic demand balance occurs.

The difficulty in finding a real sense of this term also resides in the fact that myocardial infarcts are usually smaller than the area supplied by the involved coronary artery, indicating that a viable portion of the ischemic myocardium survived without any specific therapy and may represent the border zone of the infarct. The progressive transmural extension of a small subendocardial necrotic area is a very demonstrative example in this respect.

Many observations showed that the anatomic region supplied by an occluded artery defines the area potentially at risk of infarction and not the area which is actually involved by necrosis. Ischemic bed size seems to be the major determinant of infarct size, but these two parameters are not superposable. Up to 50% of the myocardial area of a vascular bed at risk to be occluded may remain viable in the presence of an occlusion produced by a thrombus superimposed on a stenotic coronary atherosclerotic plaque, probably because this viable tissue is supplied by collateral vessels. In hearts with extensive development of these collateral vessels, the actual source of myocardial perfusion may have no obvious relationship to the anatomic distribution of the coronary arteries. In such hearts, the myocardium at risk and implicitly the border zone are very difficult to delineate since we are dealing with confluent or circumferential areas of myocardial necrosis and with a succession of recent and old infarcts in the same anatomic distribution.

The border zone might appear in certain regions of a myocardial infarct as a very important myocardial mass, acquiring a thickness of 6 to 7 mm.[160] The average risk region may represent about 40% of the left ventricular mass and the size of the true infarct may be only a small fraction of the area at risk. This still-viable myocardium at risk, which is mainly dependent on the collateral flow, is responsible for the salvage of an important mass of contractile tissue, and even in the absence of an adequate therapy it may preserve cell viability until the onset of rich capillary networks around the necrotic area. There are many data which suggest that the existence of a border zone could explain the development and progressive extension of myocardial infarcts, as well as the therapeutic reduction in infarct size.

Tridimensional observations, associated with camera lucida drawings of serially cut sections, revealed peninsulas occurring as an irregular border. The border includes a mixture of alternating viable and necrotic myocardial cells, and also regions with an abrupt transition between necrotic and apparently normal tissue. A border zone of intermediate damage was not continuously present in our material examined by means of serially cut sections, but

only in well-delineated areas. Its light microscopic feature changed with that of the necrotic area or the apparently unaltered myocardial tissue. We were able to delineate on light microscopic examination a spatial border zone, intercalated between the apparently normal and necrotic myocardium and a temporal border zone, showing different intensities of biochemical and metabolic changes. In some cases, the border zone did not represent a spatially distinct area of the ischemic region, but a time-dependent phase through which the periphery of the necrotic lesion passed. In other cases, a spatially identifiable distinct area of jeopardized myocardium could be more clearly delineated, but its limits appeared to be closely related to the evolution of the necrotic lesion. In many tissue sections we revealed changes suggesting that the annulus of myocardial tissue considered by us as a border zone may shrink in a time-dependent manner from its inner surface toward the boundary of the necrotic area. These observations support the view on the existence of a wavefront of ischemic death.[161-163]

Experimental models usually generate a sharp interface between necrotic and normal myocardium without an identifiable border zone of intermediate injury, particularly in laboratory animals with an inadequate development of collateral vessels. This may lead to the supposition that *the development of a border zone is mainly related to the existence of a well-developed collateral circulation*. The presence of anastomoses of the subendothelial plexus and intramural small arteries and arterioles could generate a distinct zone of intermediate perfusion where the progression of ischemia would be considerably slower than at the center of the necrotic area. Whereas in this center of the necrotic area cell death is inevitable (since the blood flow is totally interrupted), at the periphery of this central necrotic area, ischemia may be very mild owing to the presence of collaterals. In such peculiar conditions many myocardial cells continue to function and survive. Intermediate stages exists between these two extremes occurring as the above-mentioned border zone, where the blood flow is sufficient to prevent cell death. *Such jeopardized regions may act both as a factor of infarct size extension or as a factor of infarct size reduction*. The great tolerance of the human myocardium to ischemia compared to many laboratory animals seems to be mainly due to the presence of numerous inter- and intracoronary anastomoses and the onset of large border zones. We have often observed that in man many areas of infarction were not truly transmural, a zone of subepicardial tissue remaining apparently viable, showing the light microscopic feature of a border zone. It is tempting to presume that it received substantial collateral flow from nonoccluded vessels. The existence of these large border zones could also account for the efficacy of thrombolytic therapy in patients perfused up to 18 hr after the onset of the first symptoms of myocardial infarction.[164,165]

On the other hand, the reversible altered myocardial areas occurring as a border zone might possess chronically altered electrophysiologic properties, which may be manifested as depressed conduction and regions of unidirectional block with the potential for producing reentrant ventricular arrhythmias. This electrically deranged tissue overlying the infarct seems to be very sensitive to any further ischemia. Particularly in myocardial infarction areas produced by ischemia at a distance (ischemia in the area of distribution of a second obstructed coronary artery by interrupting collateral blood flow), these severe arrhythmias are frequently recorded. *These patients are at high risk, which is not correlated with the quantity of the necrotic myocardium, but with the quantity of the border zone of the intermediate injury*. Such patients die more often of ventricular arrhythmias and show small myocardial infarcts at autopsy.[166,167]

The following factors and mechanisms could contribute to the passage from a border zone of intermediate injury to a zone of necrosis without viable cells: coronary vasospasm, a drop in the transstenotic perfusion gradients secondary to hemodynamic alterations, hypovolemia, hypercoagulability, excessive development of atherosclerotic plaques, extension of preexisting thrombi, and steal of blood from the border zone into a vasodilated zone with an

apparently unaltered aspect. The border zone may show an augmented degree of ischemia if collateral flow decreases due to the occurrence of hypotension, such as that following arrhythmias or myocardial failure, or if left ventricular diastolic pressure rises above tissue capillary pressure. This decrease in collateral flow could transform a border zone of high collateral flow and moderate ischemia with no cell death, to a zone of low collateral flow, severe ischemia, and prompt cell death. A particular phenomenon which may also apapear in the border zone is the so-called "vascular incompetence".[168] This term is attributed to a no-reflow phenomenon due to swelling of myocardial cells and tissue edema, as well as to additional mechanisms which prevent blood flow furnished by collateral vessels to enter the border zone; this term is also employed in the available literature to designate changes which prevent blood from entering ischemic area when coronary obstruction is lysed or removed.

The border zone of human myocardial infarcts may include myocardial waves, considered to convey an ominous message: myocardial ischemia.[169] These myocardial waves seem to result from cellular injury present in or around all established myocardial infarctions; they tend to spare the myocardial and periarteriolar zones where ischemia is less severe. These fibers have been stretched and are too long for the stroma that contains them, being forced to develop multiple bands. Local mechanical conditions will determine whether adjacent fibers will bend at random, producing first order waves, or in synchrony, producing second order waves. "Waveness" of myocardial cells can be traced back to the earliest stage of infarction.[169]

E. Myocardial Salvage through Coronary Recanalization

1. General Observations

The advanced atherosclerotic plaques are usually not responsible for total occlusion of the coronary arteries, a thrombus often being superimposed on the fibrous cap of the plaque. It is this thrombus which may transform a severely but partially obstructed lesion into a totally occluded lesion. In many repeated angiographic recordings the onset of unstable angina pectoris or myocardial infarction was not associated with significant progression of the stenotic character of atherosclerotic plaques, since the main factor involved was the superimposed thrombus. Thrombus development may convert an asymptomatic lesion into an acute coronary occlusion frequently associated with anginal pain and myocardial infarction. *Whereas the stenotic atherosclerotic plaque including a variable amount of sclerohyaline tissue cannot be made to regress, the superimposed thrombus is susceptible to lysis.*

At present, coronary reperfusion or recanalization followed by myocardial salvage may be achieved by thrombolytic agents (streptokinase, urokinase, tissue plasminogen activator), coronary angioplasty, and coronary bypass grafting. Mechanical methods can be recommended when thrombolytic agents fail and surgical methods when mechanical agents fail. The use of all these procedures has undergone explosive growth during the past few years, the reopening of an occluded coronary artery after acute infarction being considered a rational way of limiting ischemic injury.

Contractile function of ischemic myocardium ceases almost immediately after coronary artery occlusion, leading to progressive myocardial cell death; therefore, a logical basis exists for the concept that coronary reperfusion during the first hours after occlusion may prevent such damage, particularly the passage from reversible to irreversible ischemic changes. In addition, reperfusion is usually associated with a reduction in myocardial infarction size. If this is not possible, reperfusion may limit the extent of infarction areas and this was detected in 75 to 80% of patients. Theoretically, the goals of early restoration of blood flow distal to the site of an occlusive lesion should be directed toward protection of the border zone that would otherwise progress to necrosis. When this protection was obtained, this could be associated with sudden relief of chest pain, resolution of ST-segment elevation on ECG, and improvement in the contraction of previously akinetic myocardium. Myocardial

salvage can also be demonstrated by the return of myocardial uptake of ^{201}Th to areas of the heart jeopardized by infarction.

Attempts have been made to establish the maximum interval from ischemia to reperfusion that would permit clinically and angiographically successful revascularization with reduction in the size of the infarct. More than 1 decade ago it was convincingly demonstrated that reperfusion of an ischemic or infarcted area of the myocardium can be successfully performed and is beneficial if carried out within 4 hr from the onset of myocardial infarction. Many results have accumulated demonstrating that reperfusion of an area of myocardium that has been ischemic for less than 2 hr in dogs or less than 4 hr in man may lead to 50% reduction in the extent of infarction, as well as improvement in cardiac functions. Reversal of clinical shock state and improvement of cardiac functions are both good indicators of a decrease in the area of nonfunctioning myocardium and hence of a decrease in the size of the infarct. There are also some revascularized areas which remain angiographically dyskinetic and electrocardiographically abnormal, but the prevalent changes would indicate a significant decrease in the size of myocardial infarction.[170,171]

The benefit of fibrinolysis might be limited if platelets, not fibrin, are the major constituents of the thrombi that obstruct the coronary arteries. Sometimes, the dissolution of a reinforcing fibrin network might favor dispersion of a mass of aggregated platelets. It is tempting to believe that platelet thrombi are intermittently formed in patients who, after thrombolytic therapy, continue to have recurrent episodes of angina. Reperfusion is not beneficial in patients in whom the vascularization of the infarction area and of the border zone has been compromised. Multiple potential causes could be involved for this no-reflow phenomenon, but plugging of capillaries by platelets or granulocytes seems to be the most frequently encountered.

Many other issues remain to be settled, related to coronary recanalization, such as:

- The maximum interval after the onset of symptoms during which the intervention can be successful
- The risk of reocclusion and reinfarction
- The risk due to bleeding and reperfusion arrhythmias
- The risk vs. benefit ratio of intracoronary thrombolysis as compared to that of the intravenous route
- The risk vs. benefit ratio of coronary thrombolysis as compared to coronary angioplasty or to coronary bypass grafting
- The risk to produce an accelerated myocardial cell disintegration in the border zone and infarction area, many injured cells swelling explosively
- Side effects obtained following dissolution of small thrombi present in coronary branch vessels and which may produce electrical disorders of the heart
- Changes in the lesion geometry during the postreperfusion period, especially of ruptured atherosclerotic plaques

In experimental models it is possible to demonstrate that intracoronary thrombi may form, disappear, and reappear over a period of minutes or hours, causing intermitent vascular obstruction. In man, such dynamic changes are not distinct enough to be visualized at the time of angiography and it is tempting to presume that they occur mainly in patients with unstable angina. It is also logical to maintain that such recurrent episodes of occlusive thrombus formation, alternating with disintegration of platelet-rich thrombi might appear in both symptomatic and asymptomatic patients with myocardial ischemia. Simply stated, we must rearrange our way of thinking and consider that *the instability of an atherosclerotic plaque may be more important than its aspect of a fixed stenotic lesion*. When thrombus formation is not followed by disintegration as a result of the development of a rich fibrin

network, clinically a passage from unstable angina to myocardial infarction is to be expected and this thrombus must be lysed to reperfuse the coronary artery. If the patient responds to streptokinase, this is clear evidence that the filling defects seen angiographically were related to coronary occluding thrombus usually developed on a stenotic atherosclerotic plaque. In such cases, the benefits depend on the interval between the onset of symptoms and the intervention: the longer the former, the less effective the latter.

The dynamics of thrombus formation and disintegration are also related to the process of reinfarction. It represents a serious complication of recanalization which may carry a mortality of up to 20%. It has been found to be associated with reocclusion of the initially recanalized coronary artery, but not every reocclusion results in reinfarction.[172] Early bypass surgery or coronary angioplasty should immediately follow, in such particular cases, streptokinase infusion. The high frequency of reinfarction in the same myocardial region as the initial infarct might be regarded as a further indirect support for the concept of a dynamic thrombus formation and disintegration. Note that the clinical course in hospitalization is more favorable in patients allocated to coronary recanalization as compared with those submitted to conventional therapy, with a lower incidence of ventricular fibrillation, pericarditis, and cardiogenic shock. On the other hand, reinfarction may appear more frequently after coronary recanalization than after conventional therapy.

Rethrombosis seems to be largely influenced by the size of the residual lumen, a major determinant of vessel rethrombosis appearing as a cross-sectional area of the lumen at the level of the residual stenosis.[173] When stenosis remains severe, an important loss of pressure persists and stasis may develop in the distal vascular segment, whereas platelets are invited to give rise to new thrombi. Unlysed thrombi may also contribute to late changes in luminal size and to new waves of platelet aggregation; the ruptured plaque probably constitutes a permanent nidus for platelet adhesion, accumulation, and degranulation. In the light of these findings, the long-term prognosis is strongly related to the possibility of converting a dynamic thrombosis into a lesion unable to give rise to new occluding thrombi.

2. Coronary Thrombolysis

As early as 1960, the feasibility of rapid dissolution of intracoronary thrombi by infusion of thrombolytic substances was demonstrated.[174] After about 20 years, attempts were made to limit in man infarct size by early reestablishment of anterograde flow.[175] Since then, many patients have been treated by early intracoronary thrombolytic substance infusion in the hope of obtaining reduction of the acute ischemic myocardial area. The patients who had successful reperfusion subsequently received oral anticoagulant treatment which has been shown to reduce complications and mortality after myocardial infarction.[176]

Streptokinase is the thrombolytic therapy most widely used in an attempt to restore coronary blood flow by lysis of the acute occluding thrombus. Streptokinase, such as that produced from cultures of Lancefield group C β-hemolytic streptoccoci, does not cleave plasminogen directly, but activates it indirectly by the formation of an intermediate. This intermediate is a stoichiometric 1:1 complex of human plasminogen or plasmin with streptokinase, able to convert plasminogen into active plasmsin.

When the insoluble fibrin networks form in coronary thrombi, it binds small amounts of a native plasma protein proenzyme, plasminogen. The thrombolytic agents currently available for clinical use are activators of the naturally occurring plasminogen-plasmin enzyme system. After infusion into the circulation both the activation of fibrin-bound plasminogen and of the circulating plasma plasminogen may result. However, whereas the activation of plasminogen to plasmin in the coronary thrombus may favor thrombus dissolution and myocardial reperfusion, the activation of plasminogen to plasmin in the circulating plasma may lead to digestion of proteins essential to normal hemostasis, thereby increasing the risk of bleeding complications. This may help to explain why significant systemic fibrinolytic activity occurs

in a high percentage of patients after streptokinase administration, but serious bleeding is uncommon.

Intracoronary infusion of streptokinase is usually begun 3 to 6 hr after the onset of symptoms of a myocardial infarction; many cardiologists recommend a rate of 2000 to 4000 IU/min for a period of 60 to 90 min. The total dose administered commonly ranges from 10,000 to 360,0000 IU, clot lysis occurring in the first 30 min of infusion. Recent work on reperfusion protocol and results in 738 patients with evolving myocardial infarction emphasizes the view that reperfusion is not a panacea for all patients with myocardial infarction; a favorable outcome depends on patient selection.[177] In these selected patients time was critical for wall motion recovery if no collaterals were demonstrated on angiography. If collaterals were present, time to reperfusion was not critical. In many works, both short-term and 1-year survival rates were significantly improved after thrombolysis, emphasis being placed on a reduction of infarct size with preservation of global left ventricular function and on regional wall motion after thrombolysis.[178]

Streptokinase is a foreign body protein with inherent problems of allergic reactions and antibody-mediated resistance. It may be replaced with a more or less similar efficacy by urokinase which seems to be less antigenic and to produce less systemic fibrinolysis. Patients receiving urokinase who are undergoing early surgery have less perioperative bleeding than a similar group of patients receiving streptokinase.[179] The great majaority of studies carried out in Europe and the U.S. use streptokinase, although in Japan, reperfusion is usually attempted with urokinase rather than streptokinase.[180]

Regardless of the thrombolytic agent used, the necessary prerequsite, coronary angiography, limits this therapy to a small proportion of the total number of patients with acute myocardial infarction susceptible to thrombolysis.

It is not possible to overlook in this field of research the existence of occasional dissenting voices assuming that coronary reperfusion may be harmful rather than beneficial. This could be mainly related to the persistence of areas of salvaged myocardium from infarction by reperfusion, but which remain severely ischemic. Such residual and severe ischemia might predispose to arrhythmias and sudden cardiac death.

The efficacy of intracoronary thrombolysis by means of streptokinase in acute myocardial infarction has been assessed in several large randomized trials. Examination of the results are impressive, since recanalization has been obtained in more than 75% of patients. On the other hand, the early hope that the intracoronary route would provide a high level concentration of streptokinase or urokinase at the level of the intraarterial clot while minimizing the predisposition to systemic bleeding was only partly realized. To surpass these difficulties, of particular value seems to be the use of human extrinsic (tissue type) plasminogen activator, a naturally occurring serine protease that activates the fibrinolytic system under physiologic conditions by converting plasminogen to plasmin. Some results would indicate that tissue-type plasminogen activators could elicit coronary thrombolysis without inducing a systemic lytic state.[181] The use of human extrinsic (tissue-type) plasminogen activators extracted from uterine tissue or from culture fluid of a cell line derived from human melanoma may represent an attractive improved therapy. This naturally occurring activator would specifically activate plasminogen that is associated with fibrin, thus localizing the formation of the fibrinolytic enzyme, plasmin, to the intracoronary obstructive thrombus. Certain investigators believe that the tissue plasminogen activator is able to produce thrombolysis followed by recanalization in less than 1 hr without bleeding complications.

An undesirable effect of reperfusion may be that certain myocardial cells pass from reversible to irreversible changes. Concepts such as the calcium and oxygen paradoxes are related to the effects of sudden reperfusion on reversible injured cells. Some of these cells remain unable to acquire an adequate metabolic activity and cardioprotective measures are recommended by means of β-blockers and calcium antagonists to support the cells or the

border zone during the reperfusion period. The attention of investigators is mainly centered on calcium ions which might become highly cardiotoxic and produce irreversible lesions if they penetrate excessively into the myocardial cells during the reperfusion period. Calcium overload damages the mitochondria so that the rate of oxidative ATP resynthesis critically diminishes and intensifies ATP consumption. The high-energy phosphate pools become exhausted and necrotic lesions may appear similar to those caused by the administration of large doses of sympathomimetic amines.[182]

The impossibility of combating a mass disease with intracoronary infusions prompted a new development, namely intravenous vs. intracoronary treatment. The favorable results of intracoronary thrombolysis and its serious limitations by requiring angiography have yearly renewed interest in the intravenous administration of thrombolytic agents. Encouraged by the promising results of intracoronary thrombolysis, certain investigators have centered their efforts on reevaluating the intravenous route.[183] They reported an encouraging 50 to 60% reperfusion rate in patients treated within 6 hr from the onset of acute myocardial infarction and studied by coronary angiography. In some studies, reperfusion of the infarct artery was demonstrated in 96% of patients,[184] and many investigators assume that the results of intravenous streptokinase therapy are comparable with those obtained using intracoronary streptokinase. Because it is difficult to have access to the specially trained personnel and expensive equipment required for intracoronary streptokinase administration, use of this procedure continues to be limited; on the other hand, systemic intravenous administration of streptokinase is simple and can be widely applied. Since salvage of jeopardized myocardium is dependent upon rapid reperfusion, the use of intravenous streptokinase may result in greater myocardial salvage; it can be started immediately upon patient identification. Attempts have been made to establish whether ultra-high dose, brief duration systemic streptokinase in intravenous infusion would lyse intracoronary clot without significant systemic bleeding.[185,186] In an analytical investigation in this field, coronary arteriography was performed before, immediately after, and 9 to 14 days after administering intravenous streptokinase (850,000 to 1.5 million IU). According to certain results, early reperfusion occurred in about 50% of patients, but in only more than $^1/_3$ was it sustained, with a significant reduction in early ventricular dysfunction and without serious bleeding.[187]

Evidence of thrombus involvement in the pathogenesis of nontransmural myocardial infarction and unstable angina is less clear both angiographically and pathologically than in acute large transmural myocardial infarction. However, certain changes have also been recorded when streptokinase was administered suggesting that *thrombosis is more frequently involved in myocardial clinical manifestations than our present methods are able to demonstrate*.[188] This indicates in addition, that dynamic changes which are not distinct enough to be visualized at the time of angiography frequently occur in coronary heart disease patients and even in those with asymptomatic myocardial ischemia. Some of these unrecorded mural thrombi that may have been present for sometime may become totally occlusive, leading to myocardial infarction and may also become organized and no longer susceptible to lysis by streptokinase. In patients with periods of recurrent attacks of unstable angina, transient diminutions of coronary blood flow may be produced either by spasm or by platelet aggregates which obstruct small intramyocardial vessels. While these observations do not delineate the main mechanisms which initiate acute myocardial infarction, they do suggest that in the first hours of the onset of symptoms, thrombolysis is required to eliminate the occlusive effects of both the occluding thrombus present in a major coronary artery and of many small microthrombi present in intramyocardial vessels. It might be assumed that it is particularly the ruptured plaque which serves as a nidus for platelet adhesion, aggregation, and degranulation, leading to thrombus formation and also to peripheral embolization. Moreover, unlysed thrombi developed on these plaques may contribute to late changes in luminal size.

3. Coronary Angioplasty

The first transcutaneous transluminal coronary angioplasty was reported about 1 decade ago,[189] the use of this method experiencing explosive growth during the last few years. Initially, only patients with single vessel disease on angiographic examination were considered for this form of therapy, but progressively the technique was extended to include patients with multivessel disease.[190,191] The method seems to be especially suited for patients with obstructive atherosclerotic plaques in the anterior descending artery. The lesion must be short, readily accessible, uncalcified, relatively concentric, and located in a rather straight arterial segment which has no important collateral vessels taking off within the area of narrowing. The method is less adequate for reopening obstructive lesions in the circumflex artery and in the intermediate segment of the right coronary artery. It is also more difficult to use in the presence of multivessel stenoses multiple successive stenoses in the same major coronary artery, or in the presence of obstructive lesions in the distal segment of the major coronary arteries, in the proximal segment of a main branch vessel, or if the obstructive lesion is located in a branch site or bifurcation area. It is, likewise, of limited use in the presence of stenoses in the left main coronary artery.

Coronary angioplasty attempts to act directly on the stenotic or occlusive atherosclerotic plaque in an effort to restore adequate coronary blood flow to the ischemic myocardium. It is regarded as a method able to give better results than those obtained by the medical treatment of consequences of myocardial ischemia and less important results than those obtained by means of revascularization surgery to bypass the coronary obstruction. Its advantages over surgery are the relative simplicity, reliability, and especially its immediate effectiveness.

Percutaneous transluminal coronary angioplasty was often added to thrombolysis in patients in which reocclusion of the infarct-related coronary artery occurred in the presence of a severe residual stenosis. The success rate often reflects the level of experience of the angiographer and the progress in instrumentation and it may augment up to 90% with a decrease of $^1/_5$ or more of the luminal encroachement produced by the stenotic atherosclerotic plaque. This is usually followed by relief of angina, improved left ventricle function, improved myocardial perfusion, and improved expertise performance.

These results demonstrate that percutaneous transluminal coronary angioplasty has gained an indisputable place in the salvage of ischemic myocardium and the economic impact of coronary angioplasty vs. coronary surgery cannot be overlooked, the major advantage of the method for the patient being that it avoids surgery. Dilatation of a stenosis in a vein graft bypass is also possible; the major contraindication is the presence of multiple stenoses in the same vascular segment.

An analytical morphologic study may not disclose any particular change or microtrauma induced by inflating the balloon under an increasing pressure of 3.5 to 6.5 atm, or may reveal fissures of the intima, ruptures of plaques, modification of plaque microarchitecture, intraplaque hemorrhage, and especially arterial dissection. The use of angioscopy seems to be of considerable importance since it may offer the possibility of detecting intraarterial changes which cannot be revealed by a coronary angiography.[192] Among the complications produced by coronary angioplasty restenosis is by far the most important and may appear in one out of every three to five cases. It remains a major problem, since it may occur within 4 to 6 hr after the procedure, despite the use of anticoagulant agents, antiplatelet drugs, and vasodilators. Many patients support a second, third, and fourth procedure, but the risk of each procedure is additive and therefore the potential advantage over coronary artery bypass surgery, if any, is minimized. In addition to restenosis, one out of every ten patients may present coronary dissection, or coronary acute occlusion with the appearance of a myocardial infarction.

Attempts are made to use pulsed and continuous laser emission to produce coronary recanalization.[193-196] The main problems are to control precisely the boundary of the tissue

injury produced by delivery of laser energy.[197] An intriguing problem is that advanced atherosclerotic involvement is characterized by the presence of diffuse stenotic lesions which usually involve the length of the proximal and intermediate segments of the major coronary arteries. This was demonstrated by our results mentioned in Chapter 4. Recent studies based on high-frequency epicardial echocardiography[198] show that diffuse atherosclerotic involvement is often present when coronary angiography detects only focal, localized lesions. The fact that coronary atherosclerosis is far more extensive than predicted by coronary angiograms must not be neglected. Likewise, it is not possible to overlook that stenotic or occlusive lesions may be located in the intermediate and distal segments of the major coronary arteries and in the proximal segment of a main branch vessel.

Because of its unique advantages of a short hospital stay, minimal discomfort, rapid convalescence, and relatively low cost, coronary angioplasty has gained great popularity, the majority of the successfully treated patients returning to work after the procedure.

IV. SUDDEN CARDIAC DEATH

A. General Observations

An "official" committee recommended that sudden, unexpected (natural) death be defined as death occurring instantaneously or within an estimated 24 hr of the onset of acute symptoms or signs.[199] In a more recent study, sudden death is defined as a witnessed or unwitnessed natural death resulting from sudden cardiac arrest occurring unexpectedly within 6 hr of a previous witnessed usual normal state of health.[200] Many other examples can be given to demonstrate that the term "sudden" is open to considerable variation in its interpretation from seconds to 24 hr. There is also considerable variation in the terms used: instantaneous death, very rapid death, rapid death, etc.

If the period when the first symptoms appear is within 30 sec to 60 min, the sudden cardiac deaths are predominantly associated with rhythm disturbances. The shorter the interval between the onset of symptoms and death, the more likely an arrhythmia is the cause. The longer the interval between the onset of symptoms and death, the more likely an acute myocardial infarction and other associated lung, liver, or cerebrovascular diseases are the main cause.

Sudden cardiac death represents a heterogeneous group of disorders with some common underlying features; the main common underlying feature is the presence of a severe coronary atherosclerotic involvement. Scar of prior myocardial alteration and especially of prior myocardial infarction are also common. When measured, heart weight is often abnormally increased; the subjects are predominantly male and risk increases with age, hypertension, heavy cigarette smoking, life stresses, obesity, and diabetes mellitus. However, none of these risk factors can be considered as a precipitating lethal event, and in many subjects the risk profile of the candidate for sudden cardiac death is more or less similar with that of the apparently healthy persons, or of the cases with clinically manifest coronary heart disease.

Sudden cardiac death may occur at many points of the natural history of coronary atherosclerosis. When all sudden cardiac deaths are indiscriminately lumped together, regardless of the point of occurrence in the course of this natural history, the pathologic features and pathogenetic mechanisms seem to be very complex and heterogeneous. In our material, the diffuse atherosclerotic involvement associated with a severe luminal narrowing was distinctly more pronounced in sudden cardiac death cases than in comparable groups of individuals dying from accidental causes. There were also isolated cases dying in traffic accidents who showed on post-mortem examination more severe coronary atherosclerotic involvement than patients diagnosed as sudden cardiac death of similar age and sex. This last remark demonstrates that when seeking to reveal how the natural history of coronary atherosclerosis relates to sudden cardiac death, it is not sufficient to assume that there were stenotic plaques

and diffuse and severe coronary atherosclerotic involvement. These stenotic plaques were present over years and their existence per se does not explain what was added in each specific case at the time of sudden cardiac death. Many victims also had myocardial lesions consistent with ischemic episodes that occurred months or years prior to death. There are no adequate data in the available literature to explain the difference between those who have severe coronary atherosclerosis and good health and those who have less severe coronary atherosclerotic involvement and are victims of sudden cardiac death. An association between advanced coronary atherosclerosis and increasing hazard of sudden cardiac death is largely accepted, but this important end stage of the natural history of coronary atherosclerosis remains a retrospective problem in which our rational approach is in large measure speculative.

Because sudden cardiac death is such a common feature of coronary atherosclerosis, there is a growing interest in obtaining further insight into the way it appears and evolves, both in the general population and in patients with myocardial clinical manifestations. *Identifying patients at increased risk for sudden cardiac death continues to be a key problem in contemporary cardiology.* Unless susceptible subjects are detected long before the catastrophic event, prophylaxis is questionable. According to certain views suggested by the results of epidemiological investigations, the contemporary epidemic of premature sudden cardiac deaths along with its underlying pathologic basis, severe coronary atherosclerosis, is due fundamentally to the evolution of lifestyle in the 20th century.[201,202] More specifically, this contemporary epidemic peculiar to industrialized countries would appear to be as a result of the inadequate evolution of nutritional and smoking habits, of sedentary living habits and arterial hypertension, and also of personal behavior patterns that become common in modern industrial society. This view is supported by the observation that more than 25% of sudden cardiac deaths occur on Monday, suggesting that lifestyle behavior and stress play significant roles.[203]

The available data would indicate that the key prevention of sudden cardiac death would appear to be an efficient alteration of the natural history of coronary atherosclerosis, particularly the reduction of the risk of unstable angina pectoris and myocardial infarction. Indeed, sudden cardiac death can be detected eight to ten times more frequently in these patients than among persons of the same age and sex with asymptomatic coronary atherosclerosis. Once overt myocardial clinical manifestations appeared in the Framingham study, the risk of sudded cardiac death escalated nine times and at this stage the major risk factors for coronary atherosclerosis and coronary heart disease have little predictive value. Also, in the Framingham study, it was revealed that in the asymptomatic population, the risk of sudden cardiac death increased with the level of both systolic and diastolic blood pressure. Even a mild degree of hypertension doubled the risk of sudden cardiac death. In over 26 years of follow-up, it has been observed that 50% of the sudden cardiac deaths in men and 70% in women occurred without prior myocardial clinical manifestations. The incidence doubled with each decade of life, women lagging behind men by 20 years.[204] The relation of sudden cardiac death with age also appeared very demonstrative in a study carried out in Helsinki. The incidence of myocardial clinical manifestations induced by coronary atherosclerosis increased from 2/100,000 at the age of 25 years to about 75/100,000 at 45 years; the incidence of sudden cardiac death cases augmented from 0/100,000 at age 25 to about 150/100,000 at 45 years.[205] This age-related increase in the incidence of sudden cardiac deaths may help to explain why in certain population groups these deaths represent up to $^1/_2$ of the total expression of coronary heart disease. In industrialized countries, it is possible to detect about 25 deaths per week from sudden cardiac death in every population of 1 million. Such deaths often occur in persons less than 65 years old, who are still in their productive years. An intriguing observation is that the proportion of sudden deaths due to coronary heart disease may be substantially greater in persons without proven overt myocardial clinical manifestations (70 to 75%) than in those with clinically recognizable coronary heart disease (25 to 30%).[206]

Some investigators implicate only extensive and severe coronary atherosclerosis as being responsible for all sudden cardiac deaths. This is an oversimplification, since sudden cardiac death also occurs in association with congestive and hypertrophic cardiomyopathies, mitral valve prolapse, valvular diseases, long Q-T syndrome, Wolf-Parkinson-White syndrome, bundle branch block, and other particular conditions. Among these particular conditions it is important to note:[207] cardioneuropathy, persistent fetal dispersion of atrioventricular node or His bundle or both, myocarditis, small vessel disease of the intramyocardial vessels and particularly of those supplying the conduction system, platelet aggregation in intramyocardial vessels, infiltrative diseases (amyloid, hemochromatosis, and sarcoid-type), and focal myocardial fibrosis. An association was revealed between autopsy evidence of myocardial bridges and sudden cardiac death.[208]

An increased incidence of sudden cardiac death was mentioned by many investigators among obese adults and this was partly related to the associated presence of arterial hypertension. It is ironic that sudden cardiac death was also revealed in high rates in obese patients which were submitted to prolonged extremely low calorie diets with rapid weight loss. The ECG pattern was similar to that of starvation and it is tempting to presume that sudden cardiac death resulted from myocardial atrophy. Deficiencies of potassium, calcium, magnesium, selenium, etc. have also been described as causing focal myocardial necrosis with myofibrillar destruction and sudden cardiac death.

Since the advent of prehospital emergency care units in the 1960s, the successful resuscitation of sudden prehospital cardial arrest substantially increased. The majority of the survivors of prehospital cardiac arrest after hospital discharge return to a normal, productive life and most of them are free of symptoms. In certain population samples, the survival outcome was found to be inversely related to the degree of proximal left anterior descending artery narrowing: at 1 year, 90% of patients with minimal narrowing were alive and asymptomatic, in contrast with 70% who had partial and 40% who had complete proximal left anterior descending artery obstruction.[209] A poor prognosis was observed with progressively more severe narrowing in the left anterior descending artery proximal to the branching site of the first septal vessel and severe dysfunction in certain left ventricular segments.

Data were also accumulated that the major risk factors for coronary heart disease are associated with sudden cardiac arrest outside the hospital and some indirect evidence is now available. Thus, for instance, cessation of smoking in survivors of out-of-hospital cardiac arrest led to a reduced risk of subsequent cardiac events, whereas no differences in survival were observed for mortality due to other causes.[210]

In individuals who collapsed in the Seattle area and who were resuscitated and brought to the hospital, two distinct evolutions could be detected:[211,212] (1) in more than 70% of cases there was no evidence of myocardial infarction and recurrent fatal arrhythmias occurred at a rate of 20%/year and (2) in less than 30% of cases there was a classic evolution toward acute myocardial infarction and recurrent episodes of sudden cardiac death occurred at a rate of only 2%/year. This tenfold difference is unexpected and suggests the existence of two different clinicopathologic entities with different pathophysiological and pathogenetic mechanisms. The Seattle experience showed that cases who were resuscitated from ventricular fibrillation without developing myocardial infarction had a worse long-term prognosis than those who had ventricular fibrillation associated with myocardial infarction. This would indicate that an arrhythmogenic milieu persisted in these patients and its presence could explain the high incidence of recurrence of cardiac arrest in such cases. This arrhythmogenic milieu[213] disappears in the infarcted area including cells which are no longer viable.

To sum up, sudden cardiac death seems to result from the contribution of at least three spheres of risk:[214] (1) severe involvement by stenotic atherosclerotic plaques of the coronary arteries, (2) myocardial electrical instability, and (3) myocardial lesions. These factors seem to develop over time in intricated chronic, subacute, and acute stages.

Since in pure cases of sudden cardiac death the main mechanisms would seem to be lethal arrhythmias and (particularly) ventricular fibrillation, a misleading premise is that one might consistently expect to find a particular pathologic counterpart of all functional disturbances. The changes resulting in sudden cardiac death may not be in certain cases clearly associated with severe lesions, particularly when a meticulous study of the conduction system and of its supplying vessels was not carried out. When such a study was performed, in virtually each heart it was possible to detect anatomic abnormalities and true lesions in the conduction tissue, its supplying vessels, cardiac nerves, and ganglia which may be involved as a potent cause of electrical instability.[215] Any simple anatomic abnormality must be interpreted in the context of other functional or anatomic changes that may augment or diminish its individual significance. It might be of interest to mention transient platelet aggregates in intramyocardial vessels, the dynamics of mural thrombi formation and disintegration, perfusion pressure, availability of collateral vessels, neural control of the heart, etc.

The following sequential events leading to sudden cardiac death have been proposed:[216]

- **Early lesions (asymptomatic stage)**: Hemodynamic factors → endothelial damage → platelet deposition → smooth muscle cell migration and proliferation → connective tissue synthesis → lipid deposition.
- **Growing lesions (angina pectoris)**: Progression of the above occurring as a slow process and organization of thrombi occurring as a rapid process.
- **Clinical complications (unstable angina)**: Plaque rupture → thrombosis → myocardial infarction. Plaque rupture could also lead to thrombotic occlusion followed by sudden cardiac death; likewise, sudden caridac death could result from changes induced by chronic myocardial fibrosis and microemboli.

The above-mentioned views, as well as many other observations which exist in the available literature, demonstrate that the old concept according to which sudden cardiac death may be regarded as a myocardial infarction before myocardial necrosis has had time to become enzymatically or pathologically manifest must be abandoned. This view has contributed to the heterogeneity of patients studied under the umbrella of acute myocardial infarction; it may also account for the frequent absence of sudden cardiac death as an anatomoclinical entity in many textbooks, monographs, and reviews.

B. Pathophysiological Data

An intriguing problem is what factors determine pump failure in some patients, an acute myocardial infarction in others, and a fatal arrhythmia in a third group, all having a more or less similar degree of coronary atherosclerotic involvement and vessel obstruction? This question requires an adequate answer for the development of preventive programs and also for effective treatments.

Sudden cardiac death is a reversible and preventable end stage of the natural history of coronary atherosclerosis, but the subject is often untreatable since he is out of the hospital. All cases with advanced coronary atherosclerosis and with chronically damaged hearts are potentially at risk to develop lethal electrophysiological phenomena occurring as severe arrhythmias, particularly ventricular fibrillation. Less emphasized are the bradycardic terminal arrhythmias, including high degree atrioventricular block, idioventricular rhythmus, marked sinus bradycardia, etc.[217] It is widely accepted that ventricular tachyarrhythmias are responsible for most cases of sudden cardiac death, ventricular fibrillation being the arrhythmia recorded in over $^2/_3$ of the victims of the time of attempted resuscitation. In certain studies, a proportion of 84% of sudden cardiac death showed ventricular fibrillation compared to only 10% bradyarrhythmia and 6% ventricular tachycardia which usually precedes ventricular fibrillation.[218] In some resuscitated patients, arrhythmias recorded at resuscitation

could be very different from those which probably triggered the cardiac arrest. On applying cardiac compression, the ventricular fibrillation may turn to ventricular tachycardia, followed by atrial fibrillation, and finally sinus rhythm. All these important electrical changes usually occur on an unchanged pattern of severe atherosclerotic involvement of the coronary arteries, as detected on repeated angiographies. This may lead to the view that for the production of various types of cardiac electrical instability there is causative interplay between many different intra- and extracardiac factors.[219]

The stimulus required to initiate ventricular fibrillation in a given situation varies from patient to patient and in the same patient from time to time. Sudden cardiac death from electrical instability of the heart is rarely caused by a single factor and of the multiple factors that may contribute, some will be sequential in nature, others occur concomitantly, and may be significant only when they appear at the same time as other factors.

Fibrillation is a chaotic, asynchronous activity of the cardiac muscle initiated by rapid impulse formation from a single focus or by reentry.[220] Primary ventricular fibrillation seems to be more likely the result of irregular impulse propagation through reentry circuits than of accelerated impulse from pacemaker fibers. The second mechanism is considered to become prevalent in secondary ventricular fibrillation once myocardial infarction has been established.[221]

Requirement for reentry are[220] slowing of conduction, unidirectional or intermittent conduction block, and lack of homogeneity of ventricular refractoriness. In addition, vulnerability to ventricular fibrillation is enhanced by large masses of contractile cells, increased automaticity of Purkinje fibers, local depolarization, and transformation of nonpacemaker into pacemaker fibers. The factors that increase vulnerability to fibrillation also include currents resulting from differences in the membrane potentials during activity or at rest.

A particular emphasis has been placed on the necessity of the existence of a large mass of modified contractile cells, the accepted hypothesis for the mechanism of ventricular fibrillation being based on studies indicating that a critical mass of myocardium is always present. This critical mass of myocardium seems to be necessary not only for the occurrence of ventricular fibrillation, but also for cardiac resuscitation. It is tempting to presume that shock causes defibrillation when it extinguishes activation fronts within a critical mass of myocardium by depolarizing all nonrefractory tissue within the critical mass.[222] More recent studies showed that a difibrillation threshold exists in each case and that successful defibrillation with epicardial electrodes requires a shock strength that reaches or exceeds the upper limit of ventricular vulnerability.[223]

The normal atrioventricular nodal His system seems to protect the heart against high ventricular rates and ventricular fibrillation by its relatively long refractory period. When this system is bypassed, as in patients with Wolf-Parkinson-White syndrome, characterized by the presence of an accessory atrioventricular pathway with a short refractory period, the risk for sudden cardiac death considerably increases. The short refractory period of the bypass tract has been held primarily responsible for allowing high ventricular rates and ventricular fibrillation.

In experimental conditions, ventricular fibrillation may result not only when a major coronary artery is occluded, but also when blood flow is restored to a previously ischemic myocardial area. The mechanism of reperfusion fibrillation was related to a rapid clearance of accumulated metabolites from ischemic areas coupled with heterogenous restoration of blood flow which may lead to electrical disorganization. In man, it is possible that relaxation of a coronary vasospasm, lysis of an occlusive thrombus, disintegration of platelet microthrombi in small intramyocardial vessels, or other events that restore coronary flow may result in ventricular fibrillation.[224] It was also recorded after thrombolysis induced by streptokinase and after coronary reperfusion induced by angioplasty when an acute relief of the occluding thrombus was obtained.

Although progress has been made in the identification of individuals at risk for sudden cardiac death, our ability to prevent successfully fatal arrhythmias are still limited by insufficient knowledge of the basic mechanisms involved in the terminal event. None of the coronary heart disease primary prevention trials, whether aimed at reduction of hypercholesterolemia in high risk men or at multiple risk factor intervention, has led to significant knowledge of the relationship between risk factors and sudden cardiac death. The effects of β-adrenergic blockade and of calcium anatagonists are worth mentioning, but of limited pathophysiological value. In the absence of such adequate data we are tempted to suggest that individuals at risk are particularly those with advanced coronary atherosclerotic plaques able to rupture or to produce occlusive thrombosis and/or microemboli in the small intramyocardial vessels. These individuals may also have an altered myocardium suffering from varying degrees of ischemia due to stenotic atherosclerotic plaques and inadequate collateral circulation. In many studies the extent of prior myocardial damage rather than acute coronary occlusion could be related to the risk of sudden cardiac death. Ventricular arrhythmias are often associated with left ventricular dysfunction, but they may appear as a risk factor for sudden caridac death independent of this dysfunction.[225]

Electrocardiographic evidence of left ventricular hypertrophy in both normo- and hypertensive individuals might be considered of prognostic significance in all attempts to prevent sudden cardiac death. This view is supported by the results of many investigations which show that left ventricular hypertrophy on the ECG may be associated with a fivefold increase in the risk of sudden cardiac death. An electrophysiological alteration exists which predisposes hypertrophied myocardial fibers to arrhythmias and arrhythmias seen in hypertrophic disorders of the heart may be caused by triggered automaticity. About 50% of cases with established hypertension have a left ventricular mass above the norm, a hyperadrenergic mechanaism being suggested, since an increased sympathetic outflow could facilitate left ventricular hypertrophy and simultaneously trigger ectopic impulse generation in the hypertrophied left ventricle. On the other hand, the proportion of sudden cardiac death cases with abormal heart weights is greater than the proportion of those with hypertension and this means that hypertension per se may predispose to sudden cardiac death by means of changes related to left ventricular hypertrophy.

Ambulatory electrocardiographic monitoring shows that in patients with coronary heart disease, ventricular fibrillation is usually preceded by ventricular tachycardia, ventricular flutter, or frequent premature ventricular complexes. Such ventricular arrhythmias are considered to arise by a prevalent reentrant mechanism on the peripheral conduction fibers of the left ventricle. They are often recorded in cases with left ventricular hypertrophy and this was related to the influence of arterial hypertension and other particular hemodynamic conditions. An increase in left ventricular mass could be demonstrated electrocardiographically in young persons 12 to 20 years old even before arterial pressure reached levels regarded as abnormal in adults.[226] Approximately 50% of cases with established hypertension had a left ventricular mass above the norm, and all these cases might be considered at risk for sudden cardiac death.

This risk increases when an augmented sympathetic activity is also present. In experimental animals, protection could be achieved with surgical or pharmacologic denervation which reduced sympathetic tone.[214]

A link is also suggested to exist between disorders of the central nervous system and ventricular fibrillation. A marked proportion of individuals who die suddenly had undergone important psychological stresses and there seems to exist a peculiar cluster of events relating to sudden cardiac death. Diverse stresses of various psychologic states provoke extensive ectopic ventricular activity which may predispose to ventricular fibrillation in susceptible cases. By stimulating the posterior hypothalamic area in dogs, the ventricular fibrillatory threshold can be lowered. On the other hand, diminished sympathetic activity augments this

threshold and a peculiar observation is that ablation of the right stellate ganglion decreased the threshold for ventricular fibrillation, whereas ablation of the contralateral stellate ganglion increased it.[227] Many other results published in the available literature indicate that the mechanisms responsible for arrhythmic death could include disturbances of both central and peripheral nervous regulation of the heart.

Coronary vasospasm is also involved in sudden cardiac death[228,229] and certain experimental studies suggest that (especially) hypomagnesemia could lead to vasoconstriction and spasm; the greater the reduction in magnesium, the greater the magnitude of the contractile response.[230]

More than 1 decade ago, it was shown that sodium arachidonate injected into the ear vein of rabbits caused sudden cardiac death and that small platelet aggregates could be detected in the intramyocardial vessels.[231] Arrhythmias, marked ST-depressison on ECG, or elevation of the ST-segment, as well as apnea preceded sudden cardiac death and no death occurred in animals pretreated with aspirin or other specific inhibitors of thromboxane synthetase.[232]

Intracoronary injection of arachidonic acid produced not only platelet aggregation in the coronary arterial tree, but also release of TXA_2 from platelets followed by spasm, occurrence of ischemic myocardial areas and sudden cardiac death. Both the mechanical obstruction produced by platelet aggregates and vasoconstriction produced by TXA_2 could be involved in the pathogenetic mechanisms of sudden cardiac death. Experimental data also revealed that *thrombus formation is a dynamic event which continues for weeks with fragmentation, embolization, and rebuilding of the platelet-fibrin microarchitecture*. During fragmentation and embolization, microinfarcts and/or lethal arrhythmias may occur. The instability of such lesions is significantly augmented if a coronary spasm appears, and the instability of the lesion itself may create a susceptible site for the occurrence of new spasms.

More than 2 decades ago experimental evidence demonstrated that emboli arising from proximal coronary thrombosis might provoke sudden cardiac death by initiating ventricular fibrillation.[233,234] An increased incidence of circulating platelet aggregates in the coronary arterial tree, an enhanced platelet activation, and release reaction all might be linked to the risk of sudden cardiac death. Microthrombi might also be produced by nonocclusive mural thrombi which consist predominantly of platelets and are embolic in origin. Theoretically, all parts of a thrombus located in the major coronary arteries or in the proximal segment of the main branch vessels may disintegrate and shower the distal microcirculation. In such cases, the administration of antiplatelet drugs reduces the incidence of both microcirculatory obstructions and arrhythmias. In clinical trials with drugs which alter platelet reactivity and which reduce platelet aggregates in the microcirculation, decreased mortality has been recorded in coronary heart disease patients, presumably through a platelet-inhibiting mechanism.[235,236]

C. Anatomoclinical Correlations

1. Significance of Coronary Thrombosis

A considerable discrepancy exists in the literature regarding the frequency of coronary occlusive thrombi in relation to sudden cardiac death and also in relation to the role that coronary thrombosis may play in its pathogenesis. The pathologist is looking for a structural explanation of what is often only a functional phenomenon, since sudden cardiac death by definition is a retrospective problem in which much speculation can be involved. Moreover, many thrombi antedate sudden cardiac death; the onset of an occlusive lesion could represent an old atherosclerotic involvement silent for years before lethal arrhythmia and compatible with a normal lifestyle. In such cases there is nothing in the past history to suggest previous manifestations of myocardial ischemia or rhythm disturbances and sudden cardiac death appears as the first clinical manifestation of coronary atherosclerosis. On post-mortem examination the pathologist can find old stenotic coronary atherosclerotic plaques complicated by thrombi, but with a long-silent clinical evolution. In such cases the real significance of

the presence of stenotic or occlusive lesions in relation to sudden cardiac death is difficult to assess. Some pathologic studies have found the incidence of occlusive coronary thrombosis to be less than 10% and an analytical investigation revealed an acute occlusive thrombosis in 15.3% of sudden cardiac death cases.[237] This reduced incidence suggests that the presence of occlusive thrombi in the coronary arteries is not the main pathogenetic mechanisms leading to sudden cardiac death. Data on the percent of coronary intimal surface covered by fatty streaks and raised lesions have no pathophysiologic, pathogenetic, and clinical use, as well as data on the degree of luminal narrowing in the absence of references to the development of the collateral circulation.

It has been postulated that among patients who die immediately or within a few minutes of the onset of symptoms, at least two different groups may be differentiated. The first group includes cases with severe and diffuse coronary atherosclerotic involvement with many stenotic plaques, but in which no new arterial events occur to explain sudden death. The prevalent sign is the electrophysiologic disturbance leading to ventricular fibrillation, cardiac arrest, and death, the pathologist being unable to find a proper morphologic explanation of the heart attack. If such cases can be resuscitated, they will not develop myocardial infarctions. The second group includes cases with acute coronary thrombosis, which could explain electrophysiological disturbances progressing quickly to ventricular fibrillation, cardiac arrest, and death. If such cases can be resuscitated, they are likely to develop myocardial infarction.[93] A similar assumption on the existence of at least two types of changes in patients who drop dead outside of the hospital has been emphasized in other studies.[238] It has been speculated that in the subjects without an occlusive coronary thrombus, infarct would not have been expected to develop; those who had an occlusive coronary thrombus, had they survived, would have had the infarct. The $^{2}/_{3}$ of cases resuscitated who do not develop myocardial infarction can probably be equated with individuals with widespread coronary stenoses; after resuscitation such cases have a poor long-term prognosis. The remaining $^{1}/_{3}$ of individuals with occlusive coronary thrombosis probably correspond to one out of every three to five individuals who develop myocardial infarction after resuscitation.[211,213,239] In point of fact, we must accept the view that the majority of patients with advanced coronary atherosclerosis who die suddenly do so without a demonstrable acute coronary thrombosis, although widespread stenotic plaques may be found. Many clinicians consider a great surprise the conclusion that there is a much lower incidence of acute coronary occlusive thrombosis in sudden cardiac death than in myocardial infarction cases. Likewise, certain pathologists consider sudden cardiac death cases without a discoverable recent event in the coronary arteries and myocardium one of the anomalies of coronary heart disease.

Many acute coronary thromboses detected in sudden cardiac death cases exhibited a layered structure, with thrombus material of different age. This would indicate that they were formed successively by repeated mural deposits and that progressive luminal narrowing developed over months or years. Also of note is the observation that many of these occlusive thrombi developed on older atherosclerotic plaques with fissures or ruptures and with intraplaque hemorrhage.[240] Diagnosis of plaque rupture was usually made when the fresh luminal thrombus contained material from the necrotic core of the plaque. Many pathologists assume that the association of organizing thrombosis with fresh thrombosis occurred after plaque rupture and reveals intricated stages of thrombus formation. Each time that organizing and acute thrombosis occurred together at the same site, the fresh lesion appeared as an extension or an addition to the older one. In certain studies it was possible to show that organizing thromboses were prevalent among those who had presented changes in clinical events 5 to 7 days before death. This would indicate that this interval might represent an approximate midpoint in the time needed to accomplish organization.[240] The significance of plaque fissuring related to the presence of thrombi is not clear, since this association has been found in 10% of control hearts, suggesting that it is not a rare event in the general population.[241]

It is thus possible to assume that plaque fissures and associated thrombosis are only more common in sudden death cases than in controls. Of particular importance is the observation that thromboses in sudden cardiac death can usually be revealed at the site of maximum narrowing by atherosclerotic plaques, that they develop in the coronary segments exhibiting the most severe degrees of narrowing.[242]

The pathogenesis of sudden cardiac death and particularly the onset of lethal arrhythmias were often associated with intermitent fragmentation of the developing thrombus, with peripheral embolization.[106,107] The period of unstable angina which sometimes precedes sudden cardiac death seems to reflect successive stages of thrombus formation and fragmentation. The presence of thrombus material as microemboli only in the terminal vascular area of a major coronary artery with an occluding thrombus, is a very demonstrative light microscopic feature of this embolization. In addition, the presence of recent microinfarcts in the myocardium supplied by the vessel with microemboli indicates that thrombus material may break off and be carried away with the coronary blood flow giving rise to myocardial peripheral embolization. As already mentioned, the existence of recurrent episodes of thrombus formation and disintegration was related to clinical manifestations occurring as unstable angina pectoris. In sudden cardiac death cases platelet microthrombi were more frequently revealed in individuals less than 45 years of age as compared with older subjects. A subgroup of sudden cardiac death cases seems to exist in younger patients in whom microcirculatory platelet thrombosis appears as the main lesion and also as a lesion with pathogenetic significance.[243-246] Experimental evidence showed that emboli arising from proximal thrombi might provoke sudden cardiac death by initiating ventricular fibrillation.[233,234] These pathologic and experimental observations require attentive investigation for platelet and fibrin microthrombi of the intramyocardial vessels in all sudden death cases, since the incidence of individuals with such particular lesions might attain more than 13%.[247] If we are dealing with patients with unstable angina pectoris suffering sudden cardiac death, this incidence might increase to 30%. A significant difference has been found between the incidence of platelet aggregates in patients with chest pain of recent onset before death (44.4%) and that in those without it (20.4%). Also, multifocal microscopic necrosis with involvement of the full ventricular thickness was significantly more common in patients with platelet emboli in intramyocardial vessels.[248] Platelet aggregates were detected in the myocardial segment immediately downstream of a major coronary artery containing an atherosclerotic plaque that had undergone fissuring and on which mural thrombus had developed. The majority of platelet aggregates showed a minimal fibrin component and were found in vessels ranging in size from arteries 1 mm in diameter up to precapillary arterioles. Since platelet emboli do not frequently contain important amounts of fibrin detectable with special stains, they often escape notice. Likewise, the examination of random sections of the myocardium will underestimate their frequency, since emboli are localized only in the area of the myocardium supplied by an artery in which an occlusive thrombosis has developed. The view that platelet aggregates in the small intramyocardial vessels represent an embolic phenomenon able to give rise to ventricular fibrillation and to produce sudden cardiac death is often mentioned in the available literature. The terminal electrical disorder might be regarded as an ischemic response to both occlusive thrombus formation and intramyocardial emboli of platelet origin.[107,215,248] By analogy with thrombi in the carotid artery and transient cerebral ischemic attacks, it is tempting to assume that thrombi of the major coronary arteries and/or of the main branch vessels could be a source of platelet emboli that are carried into the myocardium. These emboli could give rise to transient myocardial ischemia and ventricular fibrillation, followed by sudden cardiac death.

2. Significance of Associated Pathological Changes

"The diagnosis of sudden cardiac death places the pathologist in the position of being judge and jury."[249] As stated before, the pathologist is often looking for a structural expla-

nation of what is only a functional phenomenon, since sudden cardiac death by definition is a retrospective problem. The pathologic evidence of an extensive and severe coronary atherosclerotic involvement associated with myocardial damage consistent with ischemic episodes occurring months or years prior to the terminal event is largely accepted, but remains a subject of speculation. The changes leading to death may not be clearly reflected in any recognizable pathological feature on both gross inspection and light microscopy of the coronary arteries, particularly when a meticulous study of the conduction system and its supplying vessels was not carried out. James[250] maintains that "the rarity with which the cardiac conduction system is carefully examined in cases of sudden unexpected death is deplorable".

Data on the changes occurring in the vessels supplying the conduction system are presented in Chapter 2 (I.A.4, I.A.7, and I.B.4) and Chapter 4 (IV.B.2). It might be of interest to remember here that important lesions were detected in about 50% of sudden cardiac death cases in the atrioventricular node artery and in 25% of cases in the sinus node artery.[251] In addition to changes related to atherosclerotic involvement, a fibromuscular dysplasia associated with considerable luminal narrowing and media disorganization were observed.[252] Pathological changes of the vessel supplying the conduction system usually coexist with focal areas of tissue alterations in the conduction system itself.[239] Emphasis was also placed on peculiar branching anatomical patterns of the coronary arteries and on congenital cardiovascular diseases associated with hypertrophic cardiomyopathy; they could account for about 50% of the sudden death in young athletes.[253]

For didactic purposes, changes of the conduction system which may be involved in sudden cardiac death may be divided into two general categories:[250]

1. Normal variations which may favor sudden cardiac death, such as postnatal variations in the morphogenesis of atrioventricular node and His bundle
2. Pathologic changes, including diseases of the small coronary arteries, cardioneuropathy, tumors of the heart, infiltrative or inflammatory diseases, etc.

Many cases who died suddenly have no previous ECG data and in these circumstances morphological investigatins must include all the conduction tissue in an attempt to assess the significance of any abnormality found. In the presence of sudden cardiac death cases with detailed electrophysiological investigations during life, the site of an anomalous pathway can be predicted with some accuracy, allowing histological confirmation.

No post-mortem examination in patients succumbing from sudden cardiac death could be considered adequate unless a careful microscopic study of the vessels supplying the conduction system and of the conduction system itself has been performed. There is a pressing need for more detailed and more frequent study of the conduction tissue and its vessels in sudden death cases. This point has often been identified as missing in pathological reports of sudden cardiac death, in spite of the fact that it is largely accepted that automaticity derangements and a variety of reentrant mechanisms may lead to electrical instability and to lethal arrhythmias. Much of the mystique surrounding the conduction system is due to the hesitance of pathologists to investigate it. While not suggesting that lesions of the vessels supplying the conduction system, of the conductions system itself, and of cardiac nerves and ganglia can explain all sudden cardiac death cases, they cannot be ruled out as a main cause of some of these sudden death cases. In our material, the occlusion of the proximal segment of the posterior descending artery or of the first septal artery was associated to all degrees of atrioventricular blocks at the nodal level and this was also observed in other works.[254] The patients usually progress through first and second degree block to culminate in complete block, sometimes associated with syncope and sudden cardiac death. Severe arrhythmias followed by sudden cardiac death were also associated in our material with obstructive lesions in the atrioventricular node artery. Not only the remarkable location of

a great number of stenoses and occlusions in the left anterior descending artery can be associated with sudden cardiac death,[255] but also stenotic and occlusive lesions present in the vessels supplying the conduction system.

There are also studies on sudden cardiac death centered on myocardial changes and many results are presented in Section III. D.2. In addition to coagulative myocytolysis, the myocardium might show a very wide range of appearances from normal to the presence of old scarring, ventricular hypertrophy and/or small areas of acute necrosis or large areas of infarction. Hearts above 550 total weight at autopsy may have left ventricular enlargement which could be associated with sudden cardiac death in the absence of additional causative factors. In sudden cardiac death cases, the heart weight was found in excess by 500 g in more than 42% of cases and patchy left ventricular fibrosis was visualized in more than 80%.[237] A study on young men 25 to 44 years old,[256,257] revealed previous evidence of myocardial damage in 74% of cases, 62% with large myocardial scars.

The results presented in this section lead inevitably to the intriguing question: is prevention of sudden cardiac death mainly related to prevention of coronary atherosclerosis or coronary heart disease?[258] The strategy is different; the suggested possible prevention of coronary atherosclerosis demands an ambitious long-term policy mostly aimed at children, while prevention of coronary heart disease should be directed mostly toward individuals at high risk.[259] The belief that dietary fat is a cause of both coronary athereosclerosis and coronary heart disease led many to imply that a sufficiently large reduction of cholesterol and saturated fat in the diet will prevent the onset of atherosclerotic lesions and implicitly of myocardial infarction and sudden cardiac death.

There is a growing tendency to speak of reducing the risk of coronary heart disease rather than of preventing coronary heart disease. The results presented in this book do not prove a causal association between risk factors and coronary atherogenesis, but they do strongly suggest that a comprehensive risk factor prevention program can lead to a reduced coronary heart disease rate. It has been estimated that life could be extended by more than 10 years if the death attributable to coronary heart disease could be prevented.[260] This is considered a far greater extension than we could achieve by efforts directed at any other cause of death in our society.

People should be helped to make changes that promote health and reduce the risk of chronic disease and premature death.

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